THE AMERICAN JOURNAL OF THE MEDICAL SCIENCES

JULY, 1958

ORIGINAL ARTICLES

ASSOCIATION OF FUNCTIONING CARCINOID SYNDROME AND SCLERODERMA. I. CASE REPORT

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Interest in the carcinoid syndrome has heightened in recent years. This has resulted in part from the observation of additional cases, but more important in this connection has been the discovery that serotonin is the probable cause of certain manifestations of this remarkable syndrome. Several reviews of carcinoid tumors and the associated clinical features have appeared recently, including the excellent reports by Mattingly[6,7], McKusick[8], Sjoerdsma, Weissbach, and Udenfriend[13], and Ritchie[10]. Information regarding serotonin (5-hydroxyptryptamine) was thoroughly summarized by Page in 1954[9], and extended later by Sjoerdsma *et al.*,[12] and by Borges and Bessman[2]. No attempt will be made, therefore, to review again the subject literature herein.

The purpose of this and a companion report is twofold: First, to describe in detail the course and findings of a case of functioning carcinoid syndrome which also exhibited features of scleroderma; and secondly, to call attention to the potentially important implications which this association may hold with reference to the pathogenesis of scleroderma in particular and fibrosis in general.

Case Report. B.L., a 42-year-old white housewife, was first seen at the Temple University Medical Center on December 20, 1955, for the evaluation and treatment of an unusual and atypical scleroderma-like disorder. The patient exhibited marked swelling and discoloration which involved both legs and had been present for approximately 18 months. Six months earlier she had noted firmness and tightness of the skin on the inner aspect of the left thigh. By December,

1955, the skin of both legs up to the thighs had become thickened, erythematous, infiltrated and edematous.

Other points in the patient's history included sensitivity to sunlight for some 18 years. Exposure to the sun produced edema, blistering and erythema of the face. In 1953 similar changes appeared in the hands. There was, however, no fever associated with these episodes. She recalled that many years previously some nonspecific aches and pains had been present, but these had not been troublesome during the past 10 to 12 years. Of unusual interest was a marked sensitivity to alcohol. Even a taste would produce swelling of face, lips, eyes, mouth, and hands.

Noteworthy also was a history of recurrent bouts of lower right abdominal pain associated with diarrhea. She had consulted an eminent gastroenterologist for this complaint in June, 1955. These symptoms had begun abruptly about 2 months following the sudden death of the patient's father in 1946. The initial attack lasted about 3 days and was considered to be gastroenteritis. A similar episode lasting a day or so occurred a year later. Over the next 3 years there were occasional recurrences of colicky abdominal pain, some annoyance in the right lower quadrant, associated with loose, watery stools without mucus, pus or blood. For approximately 5 months in 1951 she had 10 to 20 stools per day associated with right lower abdominal discomfort. A barium meal study at that time was said to show some irritability of the duodenal cap and narrowing of the last 10 to 12 cm. of the terminal ileum. Proximal to this area was a 20 to 25 cm. long segment of ileum which was minimally dilated and exhibited serration of the margins.

On March 14, 1952, at the Hospital of the University of Pennsylvania, total hysterectomy and bilateral salpingo-oophorectomy were performed for chronic cervicitis, cervical polyp, uterine leiomyoma and bilateral paraovarian cysts. An abnormality of the terminal ileum, 4 inches from the ileocecal valve was noted, and was also inspected by a general surgeon who believed it to represent regional ileitis. There was no surgical procedure done to this area. Diarrhea and cramps developed on the third or fourth postoperative day and lasted for several weeks. Thereafter, there were several recurrences. In July, 1954, swelling and tightness of the ankles was first noted.

Physical examinations performed in June and July, 1955, revealed a tall, large-framed woman. The skin of the nose and cheeks had a deep purplish-red blush associated with numerous fine hairlike telangiectatic vessels. On the back and over the torso were several mascular, cherry red angiomata. Head, nose and throat were without abnormalities. The blood pressure was 140 systolic and 86 diastolic. The pulse was regular at 84 per minute. The breasts were normal, the lungs clear, and the heart normal in size. A grade II systolic blowing murmur was heard best over the second and third intercostal spaces to the left of the sternum. It was not transmitted to the neck or axilla. The abdomen was soft with no palpable masses or visceral enlargement. The rectal examination was negative. The extremities showed firm edematous reddish skin on both legs which pitted on pressure. Pulses were palpable and the oscillometric readings at the ankles were normal. A Rumpel-Leede's test was negative. Vasodilation tests disclosed a tendency to abnormal vasoconstriction in the feet but not in the hands. The vibratory sense was normal in the toes. Patellar reflexes were reduced and the right ankle jerk was absent.

Studies in June and July, 1955, while the patient was hospitalized at the Graduate Hospital of the University of Pennsylvania, revealed the following findings: The hemoglobin was 11 gm., and the leukocytes numbered 4,800 per c.mm. with a nonremarkable differential. Platelets were adequate. The urine analysis revealed a specific gravity of 1.026. A 1 plus albumin reaction was noted, but there was no other abnormality detected. Serologic tests for syphilis were negative. The feces were mushy, brown, and putrid and showed a small amount of mucus, but examination for occult blood, ova, and parasites was negative. No significant undigested fat, muscle or starch was noted. The serum amylase was 73 glucose units. Liver function tests including Bromsulphalein, thymol turbidity, cephalin flocculation, zinc turbidity, and serum bilirubin were all well within normal limits. Serum uric acid determinations were 6.3 mg. and 4.6 mg. per 100 ml. The blood urea nitrogen was 19 mg. and the fasting blood sugar, 103 mg. per 100 ml. The serum cholesterol was 162 mg., and the serum calcium, 9.9 mg. per 100 ml. Total proteins were 5.6 gm.%, with 3.5 gm. albumin and 2.1 gm. globulin. Gastric analysis revealed free hydrochloric acid to be present. Sigmoidoscopy to 25 cm. revealed no mucosal disease. Chest roentgenogram was also negative. An esophageal study was performed and revealed normal swallowing function. A small hiatal hernia was noted. There was marked rapidity of transit of barium through the small bowel loops. There were,

however, no satisfactory films of the terminal ileum. A barium enema disclosed no organic lesion. The patient was also seen by a dermatologist, Dr. Herman Beerman, who believed that the clinical findings were compatible with scleroderma, lupus erythematosus or poikilodermatomyositis. The cardiac findings were evaluated by a consultant who considered them to represent rheumatic heart disease with mitral and aortic valvulitis. A definite diagnosis was not reached, but an emotional etiology for the diarrhea was considered. It was also suggested that a systemic disorder, such as scleroderma, should be excluded. A bland diet, sedatives and anticholinergic agents were recommended by the gastroenterologist. Another consultant suggested that she probably had dermatomyositis and the patient was treated with Benadryl, Serpasil, and Meticorten in addition to the preceding regimen. In August, 1955, marked paroxysmal facial flushing associated with hyperhydrosis and emotional lability was noted.

The patient continued on the above program while under the care of Dr. Louis Feinstein who referred her to one of us (C.Z.). At the time of her first visit to the Temple University Medical Center, the findings were essentially unchanged from those previously noted with the exception that the diarrhea was particularly severe with 10 to 20 bowel movements daily. Furthermore, the scleroderma-like changes (Fig. 2) had ascended to involve both thighs.

The patient was begun on potassium para-aminobenzoate (Potaba), 12 gm. daily, and the Meticorten was gradually decreased and discontinued within a few days. The remainder of the program was unchanged and the patient was seen as an outpatient at 10-day intervals. Several additional studies were carried out. Four preparations for lupus erythematosus cells were negative. Platelets numbered 515,000 per c.mm. The urine creatinine excretion was 581 mg., and the creatine, 258 mg. per 24 hours. Coproporphyrins totaled 120 mg. per liter. Serum glutamic oxaloacetic transaminase levels were determined on 4 serum specimens taken over the next 2 months, and all were in the normal range. Urine analysis was negative. The cephalin flocculation test was negative. Serum proteins were 5.8 gm.%, with 4.2 gm. albumin and 1.6 gm. globulin. The gamma globulin was 3.3 units (normal being 2 to 8 units in this laboratory).

Because of the severity of the diarrhea, the patient was restudied in January, 1956. The history and findings were similar to those previously described but with the additional information that the patient was sensitive to sulfonamides and penicillin. The blood pressure was 124/84. There were purplish, raised areas on the cheeks and the nose. These were palpably thickened (Fig. 1). The skin of the hands and arms was dry, slightly discolored, and paper thin in contrast to that of the legs. There was paralysis of lateral motion of the right eye. The lungs were clear. A basal grade II systolic murmur was heard and was transmitted to the apex. Abdominal examination revealed a resistant, poorly defined mass in the right upper quadrant. It was slightly tender, but did not move with respiration and its exact nature was not clear.

Sigmoidoscopy to 20 cm. was negative. The stools showed a moderate amount of pus and undigested fibers. The barium meal was repeated and showed normal swallowing function. The esophagus, stomach, and duodenum were likewise normal. The transit time through the small bowel and colon was extremely rapid. The barium column was in the cecal area within one-half hour and in the rectum at 45 minutes (Fig. 3). The mucosal pattern of the small bowel appeared normal and there was no variation in loop caliber. The terminal ileum, ileocecal valve area, and cecum were poorly filled. The transverse colon lacked haustral markings.

In view of these findings, the bland, low residue diet was continued and anticholinergic agents, tranquilizers and Ediol were administered along with the Potaba. During the subsequent visits in January and February, there was marked improvement in the edema and the tightness of the skin of the thighs and abdominal wall was greatly lessened. However, the diarrhea persisted and exhausted the patient. She became markedly depressed, anorexic and the question of excessive sedation was raised.

On February 26, 1956, because of progressive apathy, lethargy, inappropriate response, disorientation, and restlessness, the patient was hospitalized at the Temple University Hospital. These symptoms had first been noted 2 days earlier. Physical examination revealed the blood pressure to be 110/70, pulse 80 per minute, and respirations, 18. She appeared well nourished. The malar flush and telangiectasia were of a striking purplish-red hue, and did not blanch on pressure. The skin over these areas was quite firm and thickened. The eyes were normal and no ocular or facial palsies were noted. The neck was supple, the veins were flat, and there was no lymphadenopathy. The lungs were clear to ausculation and percussion. The

heart was of normal size; a rough grade II systolic murmur was heard over the entire precordium. Macular and maculopapular areas which did not blanch on pressure were noted over the upper abdomen and chest. These were brownish-red in color. An ill-defined mass was again noted in the right upper abdomen. Peristalsis was audible. The skin was firm and tight over both legs; it was indurated and reddened, but less so than on the initial examination 2 months previously. No pedal pulses were palpable but the feet were warm.

Fig. 1.—Purplish-red, raised and palpably thickened areas on the cheeks and nose of patient on December 20, 1955.

Laboratory studies revealed the hemoglobin to be 11.4 gm. with 4.10 million red blood cells per c.mm. The hematocrit was 40% and the plasma was slightly icteric. Reticulocytes numbered 3.6%. The white blood cells totaled 7,400 per c.mm. with a normal differential. The blood platelets numbered 488,000 per c.mm.; a few giant and bizarre forms were noted on the stained blood film. A total eosinophil count was done and none were seen. Another lupus erythematosus cell preparation was negative. Urine analysis on admission gave a 3 plus albumin reaction, but the test for reducing substance was negative. The microscopic examination of the urine sediment revealed many squamous and renal epithelial cells. There were 30 to 50 white blood cells per high powered field, and 10 to 18 red blood cells per high powered field. The fasting blood sugar was 71 mg. per 100 ml. The alkaline phosphatase was 4.5 Bodansky units. The total serum protein was 4.6 gm.%, with 2.9 gm. albumin and 1.7 gm. globulin. The direct van den Bergh determination was 2.23 mg., and the indirect, 2.70 mg. per 100 ml. The thymol turbidity was 2.0 units.

The prothrombin time was 89% of normal. The serum chlorides were 330 mg. per 100 ml. The serum sodium was 127 mEq. per liter, and the serum potassium, 6.2 mEq. per liter.

The patient was placed on parenteral feedings, with added electrolytes and calcium gluconate supplements. She seemed slightly improved the following day insofar as her mental status was concerned. However, the blood pressure had fallen to 70/50 and there was generalized pitting edema, even spots to be seen, particularly over the upper extremity and trunk. Moreover, the patient was found to have a peculiar type of irregular mottled cyanosis over the head, upper extremities and trunk. The skin of the lower extremities was reddish brown in color, firm and slightly scaley. The breasts were atrophic, fibrous and multinodular. One somewhat larger nodule was noted in the left breast.

When the abdomen was opened, the liver was seen to be tremendously enlarged and

Fig. 2.—Scleroderma-like skin changes of legs of patient on December 20, 1955.

over the forehead. The urine output was extremely low. Despite vasopressors and intravenous infusions, her condition continued to deteriorate and she expired on February 28, 1956.

Permission for necropsy was obtained. Examination of the face revealed persistence of the markedly telangiectatic blood vessels over the bridge of the nose and on both malar eminences. In addition to the vasodilation, there were some frank purpuric yellow in color. It contained several irregular umbilicated white nodules with hemorrhagic halos (Fig. 4). Approximately 200 ml. of cloudy, yellow fluid were found in the peritoneal cavity. There was bilateral effusion and old apical pleural adhesions were present.

The heart weighed 300 gm. A 1.0 cm. diameter nodule of white tumor tissue was present on the lateral surface of the left ventricle. On section it was found to extend approximately half way through the sub-

Fig. 3.—Upper gastrointestinal tract roentgenographic study; 45-minute film reveals extremely rapid transit of the barium from stomach to the rectum. Arrow points to retrospective localization of primary carcinoid tumor mass.

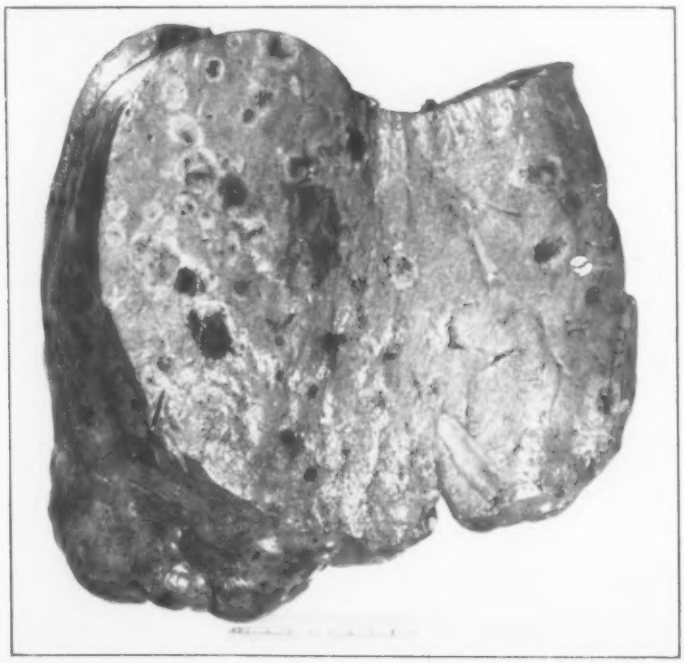

Fig. 4.—Numerous metastatic nodules of carcinoid tumor are present in the fatty liver. The centers of some are hemorrhagic.

stance of the ventricular wall. Examination of the right atrium and ventricle revealed a diffuse white endocardial sclerosis. The cusps of the tricuspid valve were thickened, nodular, white and contracted. The papillary muscles showed similar changes (Fig. 5). The cusps of the pulmonary valve were found to be glistening white in color, thickened and fibrous. They were fused together at the edges so that the pulmonary orifice was narrowed to approximately half its original diameter.

The esophagus and stomach showed no gross evidence of pathology. The upper part of the small intestine showed no evidence of disease, but further down there was a dark reddish mucosal change beginning about 70 cm. from the ileocecal valve. This change became more prominent to a point 10 cm. above the valve. At this point the intestinal wall was found to be thickened on one side. Examination revealed a nodule which was firm and white in color. It measured 2.5 cm. in diameter and appeared to

Fig. 5.—The cusps of the bicuspid valve are seen to be thickened, white, and contracted. The papillary muscles are also thickened and contracted.

Examination of the mitral valve revealed fibrous thickening of its base. The walls of the coronary arteries were slightly thickened, but there was no evidence of occlusion. The aorta showed minimal to moderate atherosclerosis. Examination of the inferior vena cava revealed that the venous wall was increased in thickness. This thickness appeared to be due to amorphous white material just under the endothelium.

The right lung weighed 400 gm. and the left lung 375. There were several white nodules up to 0.75 cm. in diameter suggestive of metastatic tumor in both lungs. These were located most frequently in the posterior portion of the lower lobe of the left lung.

lie under the mucosal surface (Fig. 6). No evidence of ulceration was present. The nodule appeared to replace the entire thickness of the bowel wall. Similar nodules were found in the periaortic lymph nodes. The bowel was found to be sharply angulated over the primary nodule in the ileum. Numerous very small brownish submucosal nodules were found in the cecum and ascending colon.

The liver weighed 4,250 gm. The capsule was thin and stretched tightly over it. The liver itself was deep yellow in color. It contained multiple umbilicated white lesions. They were surrounded by hemorrhagic halos. A large number of these nodules were seen upon section of the liver (Fig. 4).

Examination of the pancreas revealed several white nodules which were interpreted as metastatic tumors. The gallbladder and spleen showed no significant pathology grossly. The left adrenal contained a small white nodule in the pericapsular area.

The kidneys weighed 200 gm. each and were similar in appearance. Upon section, the capsules were found to be thin and stripped with ease. The entire thickness of the cortex was grayish-white in color, but the medullary areas were found to be dark gray in color and appeared intensely congested. The pelves, ureters and bladder showed no evidence of disease. The internal genitalia were absent.

Microscopic examination of sections of small bowel taken a short distance above the point of the tumor revealed epithelial denudation in the superficial parts of the villi. In the deeper portions, however, the usual small bowel columnar epithelium was present. The papilli and superficial portions of the intestine showed intense congestion and edema. A diffuse scattering of lymphocytes and neutrophils was present. The muscle coat showed no significant abnormality. Sections taken from the site of the gross intestinal tumor revealed the entire thickness of the wall to be replaced by groups of neoplastic cells with the morphologic characteristics of carcinoid tumor. These cells had round, or oval, dark to vesicular nuclei, many of which contained a small red stained nucleolus. The cytoplasm of these cells stained pink and was slightly granular in character. The cytoplasmic boundaries were indistinct. In a few places, moderate desmoplasia was noted, while in others florid groups of the neoplastic cells could be seen. Examination of other portions of the intestine near the primary tumor revealed groups of these cells infiltrating the muscularis and the subserosal coats of the bowel.

All layers of the wall of the appendix were infiltrated and partially replaced by carcinoid tumor. There was a dilation of the vessels of the submucosa, but no other pathologic change in the stomach. Groups of carcinoid tumor cells were seen in some areas of the pancreas.

Sections of the kidneys revealed no glomerular pathology. The small blood vessels were widely dilated. The epithelial cells of the proximal convoluted tubules were swollen, and had vacuolated cytoplasm. Upon staining with scarlet R, they were found to contain numerous fat droplets.

Fig. 6.—In this view of the primary tumor in the ileum, the sharp angulation of the bowel can be seen. The partially obstructed bowel proximal to the tumor is hemorrhagic and dilated.

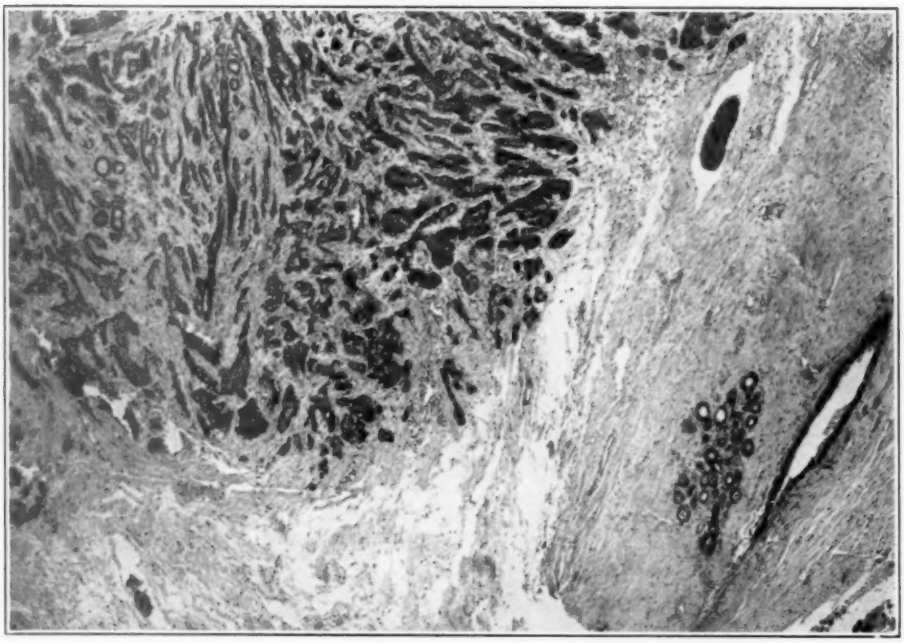

Fig. 7.—Demonstrative of the pronounced metastatic proclivity of malignant carcinoid tumors is this nodule in the substance of the breast. (× 38)

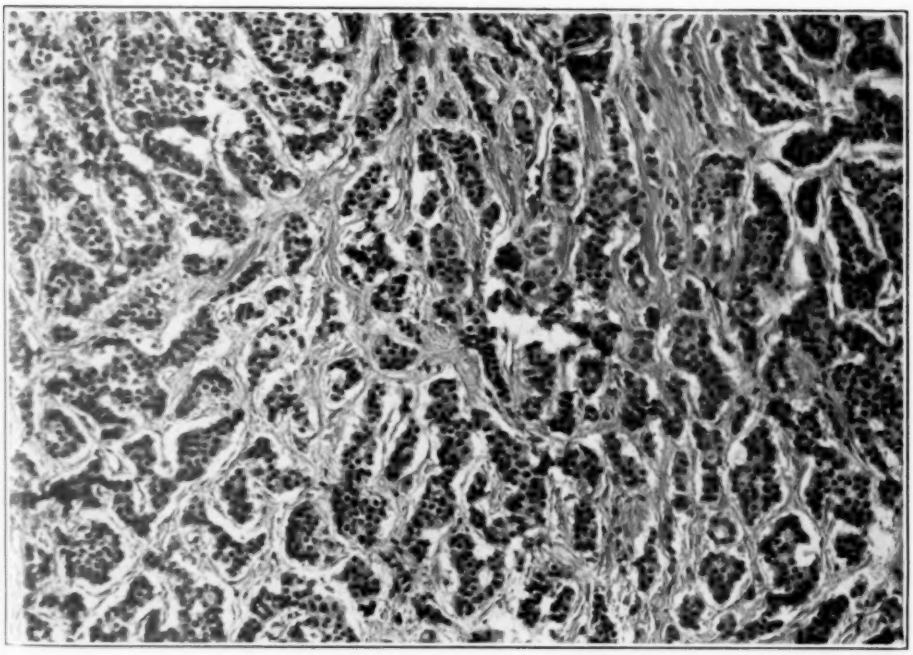

Fig. 8.—This section is a high power (× 190) view of metastatic tumor in lung. Note the considerable amount of fibrous stroma separating the islands of tumor cells.

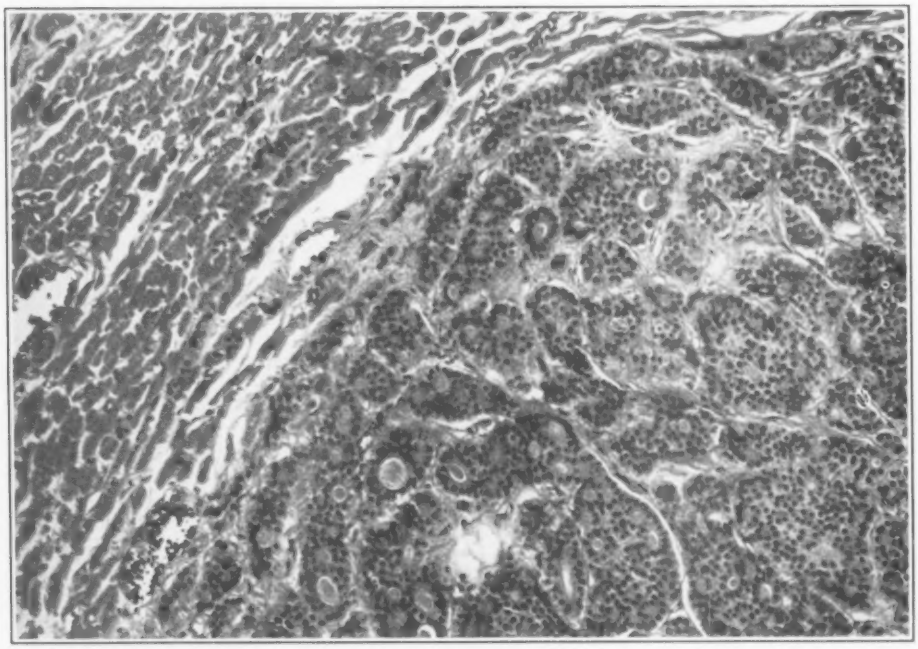

Fig. 9.—The carcinoid nodule here seen in the myocardium measured 1.0 cm. in diameter grossly. (× 190)

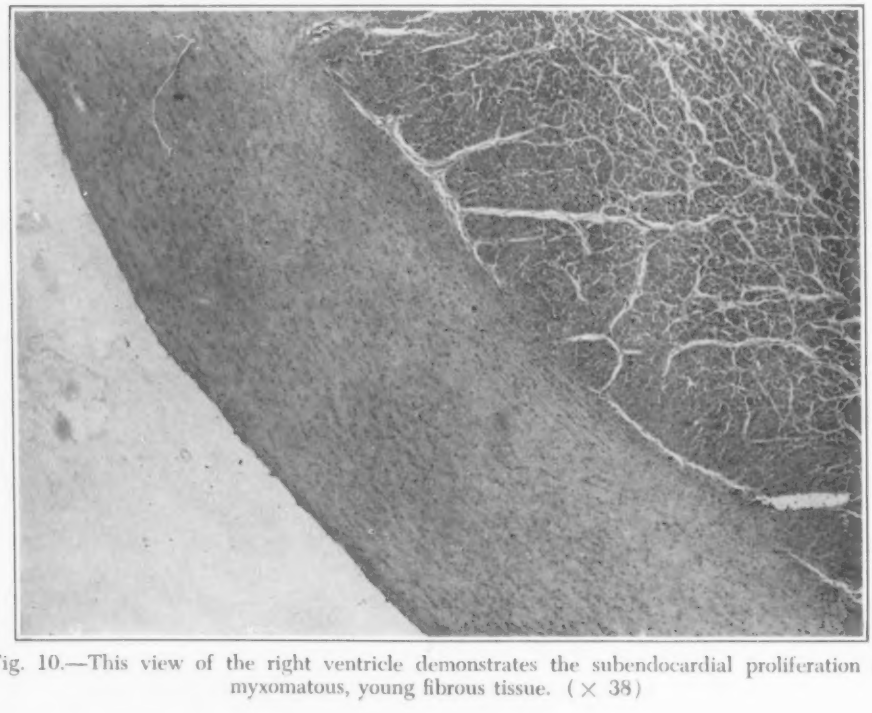

Fig. 10.—This view of the right ventricle demonstrates the subendocardial proliferation of myxomatous, young fibrous tissue. (× 38)

There was no abnormality detected on microscopic examination of the adrenals.

The nodule from the left breast was found to contain fields of carcinoid cells. The breast tissue seen in these sections had greater than usual fibrous consistency (Fig. 7).

Sections of the lungs revealed that in some places there was distention while in other areas, there was near-collapse of the alveoli. In some alveoli, evidences of edema fluid could be seen. Small islands of carcinoid cells were present in the midst of the alveolar tissue (Fig. 8).

fibrous tissue which contained a few oval to spindle-shaped nuclei (Fig. 10). The stained intercellular substance varied from eosinophilic to light blue in color. Mucinous degeneration could be seen in several places. A small nodule of carcinoid tissue was present in one area just under the endocardial surface. The myocardial fibers themselves showed no significant pathological change.

Sections of the pulmonary valve revealed it to be thickened as the result of deposition of fibrous tissue similar to that found in the subendocardial area previously described.

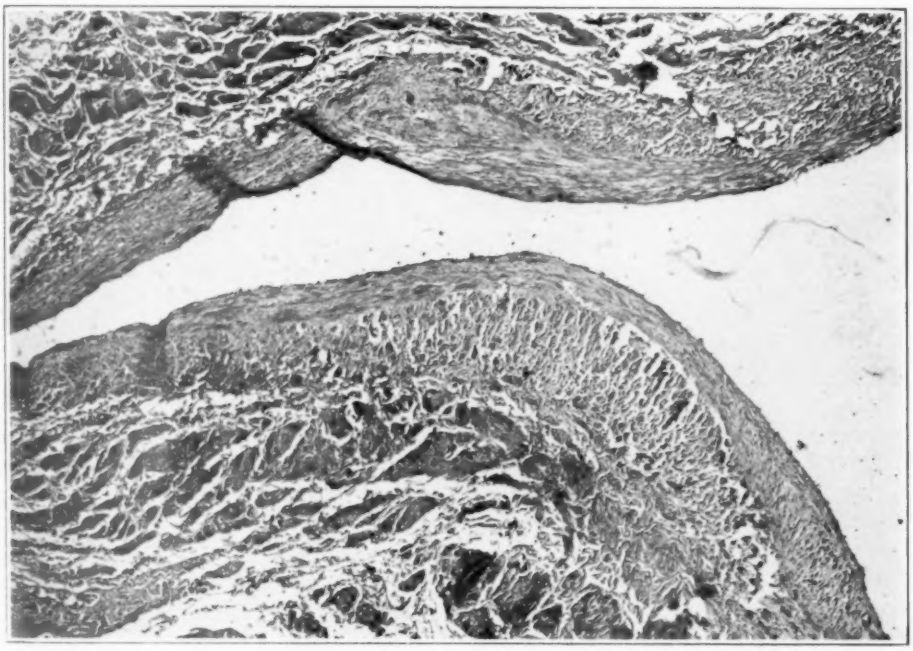

Fig. 11.—The suprahepatic portion of the inferior vena cava shows subendothelial proliferation of myxomatous fibrous tissue similar to that observed in the right heart. (× 38)

The liver sections revealed that almost all of the hepatic cells had undergone fatty change. This change appeared to be relatively uniform throughout the lobules. Also present were masses of carcinoid cells. Some of the masses showed central necrosis.

A mass of carcinoid tumor was found in one area of the heart (Fig. 9). This mass encroached upon the epicardium and nearly surrounded one small coronary artery. The wall of this artery showed no significant change. Sections showing endocardial surface revealed that the subendothelial tissue was thickened, and composed of a layer of

Sections of the mitral and tricuspid valves also revealed the same change, but to a much less degree.

Sections of the interior vena cava revealed a thin layer of fibrous tissue in the subendothelial area (Fig. 11). This tissue was similar in appearance to that seen in the endocardium.

Microscopic examination of skin sections obtained from the leg revealed the dermis to be composed of dense collagenous tissue (Fig. 12).

The cause of death was carcinoidosis.

Discussion. This patient exhibited the classical findings for carcinoid syndrome: paroxysmal flushing of the skin associated with telangiectasia; colicky abdominal pain and diarrhea; malignant carcinoid of the ileum with metastases to the liver and other organs; and valvular disease of the right heart. In addition, there was sclerodermatous involvement of the skin of the lower extremities. Despite the fact that the diagnosis is obvious in retrospect, the correct diagnosis was not made while the patient lived. The history of ileitis "confirmed" at the time of hysterectomy 4 years earlier was not only misleading, but also tragic since removal of the mass at that time may well have prevented the later course of events. Efforts to visualize a small bowel lesion were frustrated on each occasion by the extremely rapid transit of the barium meal. Again, re-examination of the roentgenograms in the light of postmortem knowledge revealed a defect which in size and location appeared to represent the tumor mass (Fig. 3).

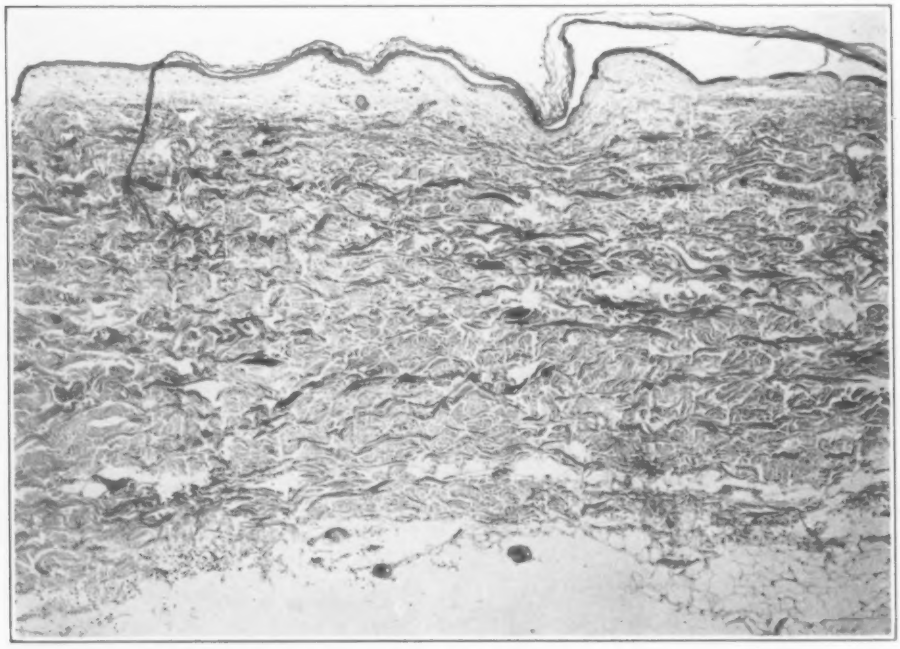

Fig. 12.—This section of skin from the leg shows the dermis to be composed of dense collagenous tissue. A moderate mononuclear infiltrate is present. (× 38)

While most of the clinical features of this case were typical of the carcinoid syndrome, there were two observations of unusual interest. One was the patient's history of extreme sensitivity to alcohol. Ingestion of only a small amount of alcohol was said to provoke generalized flushing and edema. This observation is not unique, having been noted previously in 4 other subjects with carcinoid tumors (Hanson and Serin[5] and Snow *et al.*[14]). Snow *et al.*[14] found that the administration of 10 ml. of 10% alcohol to one

of their patients was followed by flushing in 4 minutes, and the effect lasted for 15 to 20 minutes. In view of such observations it may be that sensitivity to alcohol could be employed as a clinical test in suspected carcinoidosis. To be sure, such a test would be meaningful only when the response is positive, but it would be highly suggestive when flushing is observed. Other historical clues to the presence of a functioning carcinoid tumor include the occasional provocation of generalized flushing episodes by the ingestion of food or the experience of emotional stress. Repeated attacks of colicky abdominal pain and bouts of diarrhea should, of course, also suggest the possibility of this syndrome.

The second unusual aspect of this case was the extent of fibrosis encountered. The fibrotic changes observed in the liver, inferior vena cava (Fig. 11), and right heart (Figs. 5 and 10) are those classically associated with functioning carcinoid tumors. This has been emphasized from the time of the earliest case reports (Mattingly[6,7], Rosenbaum, Santer and Claudon[11], and Thorson *et al.*[15]). A number of observers have since postulated that the sclerosis of the valves of the right side of the heart is due to an excessive amount of serotonin produced in the hepatic metastases and released into the venous circulation. Failure to induce similar alterations on the left side of the heart appears to be due to the destruction of the serotonin by monoamine oxidase present in the lungs during the pulmonary phase of blood circulation. Indeed, this concept found strong support in the studies performed by Goble, Hay, and Sandler[4]. These workers collected blood simultaneously from pulmonary artery and brachial artery in a patient with this syndrome, and found that two-thirds of the free serotonin in the blood had been removed during passage through the lungs.

In view of the well-documented propensity of metastasized functioning carcinoid tumors to produce fibrotic changes in the right heart, it is surprising that little attention has been given to the possibility that similar changes might be induced elsewhere in the body. Review of cases so far reported in the literature reveals that there have been a few instances in which fibrosis has been reported in sites other than the liver, inferior vena cava, and right heart. The most striking example was the patient reported by Cassidy[3] in 1931. Aside from the characteristic clinical syndrome, this patient had two findings of interest. There was a "hard edema of the legs" and an impassable stricture a few inches above the anus. At necropsy, the pelvic organs could be removed only with great difficulty: "It looked as though the pelvis had been filled with plaster of Paris." Tissue removed from the pelvis yielded the astonishing finding of extensive fibrosis without evidence of malignancy.

Bean, Olch, and Weinberg[1] found "much sclerosis together with atrophy" in the skin of one of their cases, but did not elaborate further on this finding.

In the present case, not only is there marked change to be seen in the skin (Fig. 12), but an increase in fibrotic elements was noted in the metastatic tumor sites (Figs. 7, 8 and 10). To be sure, sclerosing effects are exhibited by other tumors, but this does not necessarily militate against the possible causal relationship of the carcinoid metastases and the fibrotic changes associated with them. The presence of extensive cutaneous changes resembling those found in scleroderma[16], along with the other manifestations of

fibrosis in this case, suggests the possibility that all of these findings might be directly due to the functioning carcinoid tumor. The association of these findings has been interpreted by Zarafonetis[17] as a possible clue to the pathogenesis not only of the fibrotic changes of the functioning carcinoid syndrome, but also of scleroderma and other fibrotic states. It has been further theorized that such fibrotic changes result from a deranged interaction of the serotonin-monoamine oxidase mechanisms at the tissue level. This concept is presented and discussed in detail in a companion article[17].

Summary. A case of functioning carcinoid syndrome has been described. During life, this patient exhibited paroxysmal flushing of the skin, associated with telangiectasia; colicky abdominal pain and diarrhea; cardiac murmurs; and scleroderma-like changes of the lower extremities. Attacks of flushing and edema could be precipitated by the ingestion of alcoholic beverages or by emotional stress.

Necropsy revealed malignant carcinoid of the ileum with metastases to the liver, lungs, heart, breast, and appendix. Fibrotic changes were observed not only in the liver, inferior vena cava, and right heart, but also in the skin of the legs, and in association with the tumor metastases. The possible implications of this observation have been discussed.

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(Interlingua on page 23)

NICKEL POISONING

VII. THE THERAPEUTIC EFFECTIVENESS OF ALKYL DITHIOCARBAMATES IN EXPERIMENTAL ANIMALS EXPOSED TO NICKEL CARBONYL*

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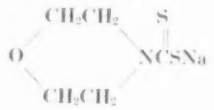
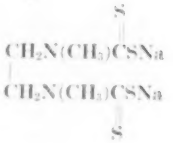
For several years our laboratory has studied the hazards of exposure to nickel carbonyl[9,10,13,17]. In the course of these investigations several agents, including Dimercaprol and calcium disodium ethylenediaminetetracetic acid, were tested for their therapeutic effect following exposure. It was shown that Dimercaprol increased the LD_{50} value of rats exposed to nickel carbonyl by a factor of approximately two[9]. On the other hand, $CaNa_2EDTA$ was found to be ineffective as an antidote in experimental animals[17]. More recently our attention has been directed to investigations of other promising chemotherapeutic agents. This paper is concerned primarily with the antidotal properties of the *dithiocarbamates* following exposure of experimental animals to inhalation of nickel carbonyl. Studies on Antabuse and Penicillamine are also reported.

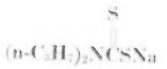
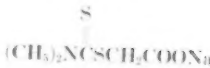
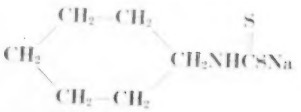
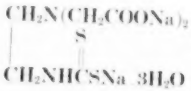
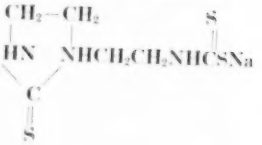
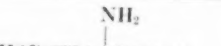
The dithiocarbamates are widely used as reagents for the analyses of certain metals (Welcher[16]). The sodium salt of the diethyldithiocarbamate is used as a specific reagent in our laboratory for the measurement of nickel in biological fluids (Kincaid *et al.*[10]). Hald, Jacobsen and Larsen have shown that this dithiocarbamate is relatively nontoxic for mice and rats[7]. Furthermore, these workers reported the LD_{50} value by parenteral injection to be approximately 1.5 gm. per kg. of body weight, a value recently confirmed in our laboratory.

In considering the chemotherapeutic properties of the dithiocarbamates, it may be noted that the bismuth salt of diethyldithiocarbamate was found to protect mice against Type I pneumococcal infections when given in intramuscular doses of 500 mg. per kg. of body weight (Goth and Robinson[5]). Moreover, in a study of the antituberculous activity of a series of chelating agents sodium diethyldithiocarbamate was observed to be an effective inhibitor for the growth of Mycobacterium tuberculosis (Garattini and Leonard[4]). Recognition and correlation of these chemical and biological properties of the dithiocarbamates form the foundation for the present investigation.

*This study was supported in part by contract of Jefferson Medical College with the Atomic Energy Commission.

TABLE 1.—COMPARISONS OF THE ACUTE TOXICITY AND THE ANTIDOTAL ACTIVITY OF DITHIOCARBAMATES IN NICKEL CARBONYL POISONING IN MICE

Compound	*Formula*	*Intraperitoneal LD_{50} mg./kg. (Approx.)*	*Concentration of $Ni(CO)_4$ mg./L.*	*Protection Against $Ni(CO)_4$—%*	*Parenteral Dosage mg./kg.*
Sodium diethyldithiocarbamate ("Dithiocarb")	$(C_2H_5)_2NCSNa$	1500	.09	100	50
Sodium dimethyldithiocarbamate	$(CH_3)_2NCSNa$	<1000	.09	100	50
Sodium diisopropyldithiocarbamate	$(iso\text{-}C_3H_7)_2NCSNa$	>1000	.09	100	50
Sodium morpholine-4-dithiocarboxylate		1000	.09	100	100
Sodium N,N′-ethylene-bis-dithiocarbamate ("Dithane A–10")		>500	.09	50	250
Sodium 2-(2-oxo-1-imidazolidyl)ethyl-dithiocarbamate		<1500	.09	50	100
Sodium N,N′-dimethyl-N,N′-ethylene-bis-dithiocarbamate		>1500	.09	40	100

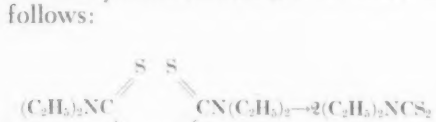
Sodium di-*n*-propyldithiocarbamate		<1000	.09		
Carboxymethyl N,N-dimethyldithiocarbamate, sodium salt		1500	.09	0	100
Sodium cyclohexyldithiocarbamate		<500	.09		
Trisodium 2-[bis-(carboxymethyl)amino]-ethyldithiocarbamate trihydrate		<1000	.09	0	100
Sodium 2-(2-thiono-1-imidazolidyl)ethyl-dithiocarbamate		<1000	.09		
Sodium methyldithiocarbamate		<500	.09		
Tetræthylthiuram disulfide ("Antabuse")		>500	.09	70	250
β,β-dimethylcysteine ("Penicillamine")		250 (Beyer, 1957)	.09	100	200 mg./kg. (100 stat & 4 h.) 250 mg.—fatal

Method. Nickel carbonyl was administered to experimental animals by inhalation in an exposure chamber recently described by Sunderman and his coworkers[14]. The chemical was vaporized from a solution of nickel carbonyl dissolved in a mixture of equal parts of ethyl alcohol and ether. Continuous measurements of the concentration of nickel carbonyl present in the chamber were made throughout the 30-minute periods of exposure, by methods previously described (Kincaid *et al.*[10]).

A series of exposures to nickel carbonyl was undertaken with C–57 strain mice and Wistar strain rats. Following each exposure, the animals were divided into 2 groups of 10 each. One group served as the nickel carbonyl control and the other group was immediately given test doses of various dithiocarbamate derivatives intraperitoneally. Mortality ratios were determined 3 and 5 days after the exposure.

Results. ANTIDOTAL ACTIVITY OF A SERIES OF DITHIOCARBAMATES. In Table 1 are presented the chemical names and formulas, the approximate LD_{50} values, and the relative antidotal effectiveness of dithiocarbamates in acute nickel carbonyl intoxication in mice. It will be seen in Table 1 that sodium diethyldithiocarbamate, sodium dimethyldithiocarbamate, sodium diisopropyl dithiocarbamate and sodium morpholine dithiocarboxylate appear to be the most effective of the compounds investigated. Of these 4, sodium diethyldithiocarbamate is the least toxic. For brevity the term "Dithiocarb" will be used in place of sodium diethyldithiocarbamate in the remainder of the paper.

ANTIDOTAL ACTIVITY OF ANTABUSE. Antabuse (Tetraethylthiuram disulfide) has been used in the treatment of chronic alcoholism. Eldjam[3] has shown that this compound is reduced to diethyldithiocarbamate *in vivo* as follows:



Antabuse — Diethyldithiocarbamate

The oral LD_{50} value for Antabuse in the rat has been reported to be 8.6 gm. per kg. of body weight (Child and Crump[2]). No symptoms of toxicity were observed after a single intraperitoneal dose of 0.6 gm. per kg. given in an aqueous suspension (Hald, Jacobsen and Larsen[6]). In view of the relation of Antabuse to diethyldithiocarbamate, preliminary experiments were undertaken to ascertain whether or not Antabuse possessed any antidotal properties in the treatment of nickel carbonyl poisoning. In Table 1 it will be seen that Antabuse administered intraperitoneally in a dosage of 250 mg. per kg. of body weight does provide limited protection against the acute toxic effects of nickel carbonyl. However, since the dosage required to produce this effect is several times greater than that required for several other dithiocarbamates, no further studies with Antabuse were undertaken.

ANTIDOTAL ACTIVITY OF PENICILLAMINE. Penicillamine (dimethylcysteine), has been reported to enhance the urinary excretion of copper following its oral administration to patients suffering from Wilson's disease (Walshe[15]). The compound is reported to be relatively nontoxic and rapidly excreted by the kidney. The results of our studies indicate that Penicillamine in a dosage of 100 mg. per kg. of body weight administered intraperitoneally immediately following exposure and repeated 4 hours later provides protection against the acute toxic effects of nickel carbonyl (Table 1). However, if the dose of Penicillamine is increased to 250 mg. per kg. of body weight all of the animals died within 6 hours after the exposure. Since the lungs of these animals were not hemorrhagic (as is seen in acute nickel carbonyl poisoning) and since death from nickel carbonyl in the concentration employed does not usually oc-

cur until 48 to 72 hours after exposure, it is believed that death was due primarily to the toxicity of Penicillamine (Beyer[1]).

"DITHIOCARB" AS AN ANTIDOTE FOR NICKEL CARBONYL IN MICE. The results given in Table 2 demonstrate that "Dithiocarb" in dosages of 50 and 100 mg. per kg. of body weight administered intraperitoneally provides complete protection against the otherwise lethal effects of acute exposure to nickel carbonyl in concentrations many times the LD_{50} value.

"DITHIOCARB" AS AN ANTIDOTE FOR NICKEL CARBONYL IN RATS. The results presented in Table 3 indicate that "Dithiocarb" in a dosage of 100 mg. per kg. administered intraperitoneally to rats provides complete protection against exposure to nickel carbonyl in concentrations ranging from 0.50 to 1.99 mg. per L. for a period of 30 minutes.

TABLE 2.

A. EFFECT OF DITHIOCARB ADMINISTERED INTRAPERITONEALLY IN A DOSAGE OF 100 MG. PER KG. OF BODY WEIGHT IN MICE EXPOSED TO VARIOUS CONCENTRATIONS OF $Ni(CO)_4$

Control and Treated Groups (10 each)	*Nickel Carbonyl mg./L.*	*Mortality Ratios 3 Days*	*Mortality Ratios 5 Days*
$Ni(CO)_4$	0.048	4/10	7/10
$Ni(CO)_4$ + Dithiocarb	0.048	0/10	0/10
$Ni(CO)_4$	0.06	8/10	10/10
$Ni(CO)_4$ + Dithiocarb	0.06	0/10	0/10
$Ni(CO)_4$	0.075	7/10	10/10
$Ni(CO)_4$ + Dithiocarb	0.075	0/10	0/10
$Ni(CO)_4$	0.12	6/10	10/10
$Ni(CO)_4$ + Dithiocarb	0.12	0/10	0/10
$Ni(CO)_4$	0.18	7/10	10/10
$Ni(CO)_4$ + Dithiocarb	0.18	0/10	0/10
$Ni(CO)_4$	0.30	10/10	10/10
$Ni(CO)_4$ + Dithiocarb	0.30	0/10	0/10

B. EFFECT OF DITHIOCARB ADMINISTERED INTRAPERITONEALLY IN A DOSAGE OF 50 MG. PER KG. OF BODY WEIGHT IN MICE EXPOSED TO VARIOUS CONCENTRATIONS OF $Ni(CO)_4$

Control and Treated Groups (10 each)	*Nickel Carbonyl mg./L.*	*Mortality Ratios 3 Days*	*Mortality Ratios 5 Days*
$Ni(CO)_4$	0.048	9/10	9/10
$Ni(CO)_4$ + Dithiocarb	0.048	0/10	0/10
$Ni(CO)_4$	0.06	10/10	10/10
$Ni(CO)_4$ + Dithiocarb	0.06	0/10	0/10
$Ni(CO)_4$	0.12	10/10	10/10
$Ni(CO)_4$ + Dithiocarb	0.12	0/10	0/10
$Ni(CO)_4$	0.18	10/10	10/10
$Ni(CO)_4$ + Dithiocarb	0.18	0/10	0/10
$Ni(CO)_4$	0.30	10/10	10/10
$Ni(CO)_4$ + Dithiocarb	0.30	0/10	0/10

The mortality ratios obtained when only 50 mg. of "Dithiocarb" per kg. of body weight were administered to rats following exposure to concentrations of nickel carbonyl ranging from 0.50 to 1.99 mg. per L. for 30 minutes are given in Table 3. The lower dosage of the antidote provides the same protection as was observed with the 100 mg. dose. It should be pointed out that in the groups exposed to 1.99 mg. per L. of nickel carbonyl many of the rats died while still in the exposure chamber. The remainder were moribund upon removal from the exposure chamber but all the animals administered "Dithiocarb" survived. These findings, in addition to those obtained with mice, further substantiate the dramatic protective effect of "Dithiocarb" in nickel carbonyl intoxication.

DELAYED TREATMENT OF NICKEL CARBONYL POISONING WITH "DITHIOCARB" IN MICE. The studies presented thus far have shown the protective action of "Dithiocarb" in acute nickel carbonyl poisoning when administered immediately following exposure. Studies were undertaken to determine the effectiveness of "Dithiocarb" in the delayed treatment of acute nickel carbonyl intoxication. The procedure employed was similar to that previously outlined with the exception that 30 mice were exposed simultaneously to nickel carbonyl in concentrations of 0.12 mg.

TABLE 3.

A. EFFECT OF DITHIOCARB ADMINISTERED INTRAPERITONEALLY IN A DOSAGE OF 100 MG. PER KG. OF BODY WEIGHT IN RATS EXPOSED TO VARIOUS CONCENTRATIONS OF $Ni(CO)_4$

Control and Treated Groups (10 each)	*Nickel Carbonyl mg./L.*	*Mortality Ratios 3 Days*	*Mortality Ratios 5 Days*
$Ni(CO)_4$	0.50	6/10	7/10
$Ni(CO)_4$ + Dithiocarb	0.50	0/10	0/10
$Ni(CO)_4$	0.79	7/10	9/10
$Ni(CO)_4$ + Dithiocarb	0.79	0/10	0/10
$Ni(CO)_4$	1.26	9/10	10/10
$Ni(CO)_4$ + Dithiocarb	1.26	0/10	0/10
$Ni(CO)_4$	1.99	10/10	10/10
$Ni(CO)_4$ + Dithiocarb	1.99	0/10	0/10

B. EFFECT OF DITHIOCARB ADMINISTERED INTRAPERITONEALLY IN A DOSAGE OF 50 MG. PER KG. OF BODY WEIGHT IN RATS EXPOSED TO VARIOUS CONCENTRATIONS OF $Ni(CO)_4$

Control and Treated Groups (10 each)	*Nickel Carbonyl mg./L.*	*Mortality Ratios 3 Days*	*Mortality Ratios 5 Days*
$Ni(CO)_4$	0.50	6/10	6/10
$Ni(CO)_4$ + Dithiocarb	0.50	0/10	0/10
$Ni(CO)_4$	0.79	4/10	7/10
$Ni(CO)_4$ + Dithiocarb	0.79	0/10	0/10
$Ni(CO)_4$	1.26	8/10	10/10
$Ni(CO)_4$ + Dithiocarb	1.26	0/10	0/10
$Ni(CO)_4$	1.99	10/10	10/10
$Ni(CO)_4$ + Dithiocarb	1.99	0/10	0/10

per L. for 30 minutes. They were then divided into 3 groups of 10 each as follows: Group A received no therapy, Groups B and C received "Dithiocarb" at varying intervals after exposure (Table 4).

The results given in Table 4 indicate that the withholding of "Dithiocarb" for 4 and 8 hours after exposure provides the same order of protection as observed when the "Dithiocarb" was administered immediately after exposure. However, delaying treatment until 24 hours after exposure failed to provide the same protection.

TABLE 4.—EFFECT OF DELAYED PARENTERAL ADMINISTRATION OF DITHIOCARB IN RATS FOLLOWING EXPOSURE TO NICKEL CARBONYL

Control and Treated Groups	*Nickel Carbonyl mg./L.*	*Mortality Ratios 3 Days*	*Morality Ratios 5 Days*
A. $Ni(CO)_4$	0.12	10/10	10/10
B. $Ni(CO)_4$ + Dithiocarb, 50 mg./kg. I.P. after 4 h.	0.12	1/10	1/10
C. $Ni(CO)_4$ + Dithiocarb, 100 mg./kg. I.P. after 4 h.	0.12	1/10	1/10
D. $Ni(CO)_4$	0.12	10/10	10/10
E. $Ni(CO)_4$ + Dithiocarb, 50 mg./kg. I.P. after 8 h.	0.12	0/10	0/10
F. $Ni(CO)_4$ + Dithiocarb, 100 mg./kg. I.P. after 8 h.	0.12	0/10	0/10
G. $Ni(CO)_4$	0.12	8/10	10/10
H. $Ni(CO)_4$ + Dithiocarb, 50 mg./kg. I.P. after 24 h.	0.12	4/10	4/10
I. $Ni(CO)_4$ + Dithiocarb, 100 mg./kg. I.P. after 24 h.	0.12	6/10	7/10

TABLE 5.—EFFECT OF DITHIOCARB ADMINISTERED ORALLY TO RATS EXPOSED TO LETHAL CONCENTRATIONS OF NICKEL CARBONYL

Control and Treated Groups	*Nickel Carbonyl mg./L.*	*Mortality Ratios 3 Days*	*Morality Ratios 5 Days*
$Ni(CO)_4$	0.50	5/10	9/10
$Ni(CO)_4$ + Dithiocarb, 50 mg./kg. orally	0.50	2/10	3/10
$Ni(CO)_4$ + Dithiocarb, 100 mg./kg. orally	0.50	1/10	1/10
$Ni(CO)_4$	1.99	10/10	10/10
$Ni(CO)_4$ + Dithiocarb, 50 mg./kg. orally	1.99	7/10	10/10
$Ni(CO)_4$ + Dithiocarb, 100 mg./kg. orally	1.99	4/10	6/10

THE ORAL ADMINISTRATION OF "DITHIOCARB" IN ACUTE NICKEL CARBONYL POISONING IN RATS. The LD_{50} value of "Dithiocarb" by oral administration in rats was found to be approximately the same as the value of 1.5 gm. per kg. by parenteral administration. Studies were undertaken to determine the therapeutic efficacy of "Dithiocarb" administered orally to rats exposed to nickel carbonyl. The procedure followed was similar to that previously outlined with the exception that "Dithiocarb" was administered orally by stomach tube immediately following the exposure.

It will be seen in Table 5 that "Dithiocarb" given orally in dosages of 50 and 100 mg. per kg. of body weight provides moderate protection against the acute toxic effects of nickel carbonyl, particularly at the lower levels of exposure. However, oral administration of "Dithiocarb" does not provide the same order of protection as parenteral administration.

NICKEL METABOLISM FOLLOWING EX-

POSURE TO NICKEL CARBONYL AND TREATMENT WITH "DITHIOCARB." Nickel balance studies were undertaken in rats exposed to nickel carbonyl. Twenty-four female albino rats weighing 125 to 150 gm. were used in this metabolic study. The animals were housed individually in metabolism cages fitted with silicone-coated glass funnels to aid in the collection of urine. Plastic mesh screening was placed between the bottom of the metabolism cages and the funnels used to collect the feces. The animals were fed a diet of powdered Purina Dog Chow Checkers and given distilled water *ad libitum*. At the beginning of each metabolic period the time was noted, the food weighed, and the bladder emptied by gently pressing the lower abdomen of the rat. The urine thus obtained was discarded. At the end of each metabolic period the time was again noted, the food weighed and the bladder emptied. The weights of ingested food and excreted stool, and the volumes of urine were obtained for each metabolic period. The stools were pooled, dried to constant weight and stored in a desiccator. Analyses for nickel were made on aliquot portions of the food, feces and urine.

The night before exposure the food was taken away from the rats and the next morning the animals were placed in the exposure chamber and subjected to an inhalation of nickel carbonyl at a concentration of 0.25 mg. per L. for 30 minutes. At intervals during the exposure period the concentrations of nickel carbonyl in the chamber were determined by direct analysis.

Twenty-four rats were exposed to nickel carbonyl. Twelve of these rats served as controls and the remaining 12 received "Dithiocarb" intraperitoneally in a dosage of 100 mg. per kg. of body weight immediately after exposure. The mean values for the nickel balance studies in these animals are presented in Fig. 1. The data indicate that "Dithiocarb" increases the excretion of nickel in rats exposed to nickel carbonyl by approximately 20% in the first 3-day period following exposure ($P = 0.05$) (Snedecor[11]). Attention is directed to the more than threefold increase in food consumption and excretion of nickel in the exposed animals receiving "Dithiocarb" as compared to the exposed animals which were untreated. These studies give further evidence of the antidotal activity of this compound in combatting acute nickel carbonyl poisoning.

THE EFFECT OF "DITHIOCARB" ON THE CONCENTRATION OF BLOOD GLUCOSE IN RABBITS. Kadota and Midorikawa[8] reported that the intravenous administration of sodium diethyldithiocarbamate in a dosage of 500 to 1000 mg. per kg. of body weight to rabbits produced immediate hyperglycemia. Subsequently, some of the rabbits developed hypoglycemia. Histologic examination of the pancreatic glands of these animals was reported to give evidence of degenerative lesions of the alpha cells with slight degeneration of the beta cells. The dosages of "Dithiocarb" employed by Kadota and Midorikawa were several times greater than the effective doses employed in our present studies. Nevertheless, the studies by the Japanese workers led us to measure the concentration of blood glucose in rabbits receiving "Dithiocarb" and to study the effects of "Dithiocarb" on the pancreas of sacrificed rabbits.

The concentrations of blood glucose were measured in samples of blood of 9 normal rabbits by the Sunderman micro method[12]. Immediately following the withdrawal of blood the rabbits were given "Dithiocarb" intravenously in dosages of 100 and 250 mg. per kg. of body weight. Two hours later, blood samples were again taken for analyses.

This procedure was repeated in some of the animals at intervals of 24, 48 and 96 hours.

The results which are presented in Table 6 indicate that the intravenous administration of "Dithiocarb" in dosages of 100 and 250 mg. per kg. of body weight respectively, produces hyperglycemia 2 hours after administration. However, at both dosage levels the concentrations of blood glucose in the treated animals return to normal levels within 48 hours. Since "Dithiocarb" combines with copper, the possibility is entertained that the increased values for blood glucose may be artifactitious and due to increased reduction of copper reagent in the measurement of blood glucose. Histopathologic examinations of the pancreas of some of the rabbits failed to reveal any noteworthy change in either the alpha or beta cells.

Discussion. The mechanism by which "Dithiocarb" counteracts the acute toxic effects of exposure to nickel carbonyl is speculative. The dithiocarbamates are strong complexing agents which are used for the detection of cadmium, cobalt, copper, lead, manganese, mercury, strontium and nickel[16]. On the basis of this chelating action it is a reasonable assumption that "Dithiocarb" combines with the nickel in the tissues of animals poisoned with nickel carbonyl to form a nontoxic

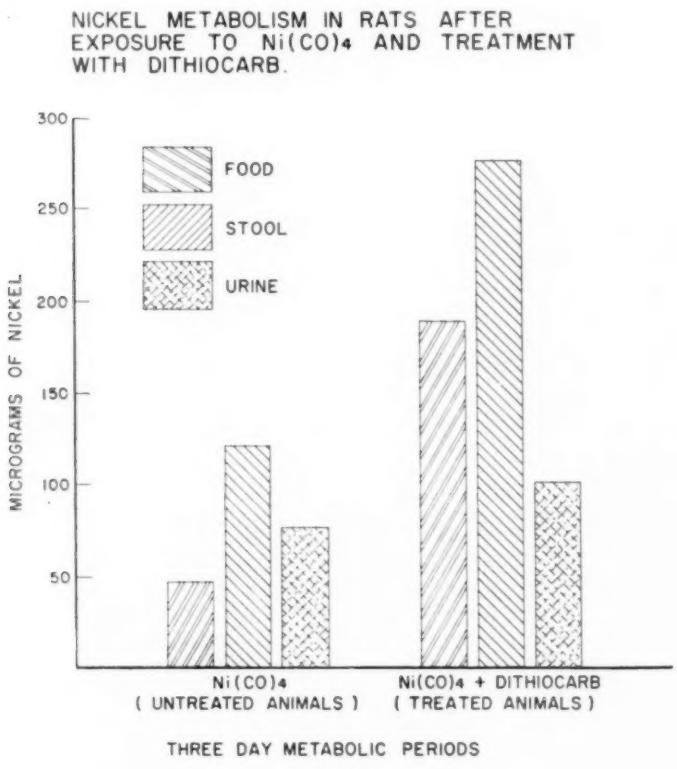


Fig. 1

chelate. It is noteworthy that "Dithiocarb" and closely related derivatives are effective while other chelating agents, such as calcium disodium ethylenediaminetetracetic acid (West and Sunderman[17]), are ineffective.

Our metabolism studies suggest that "Dithiocarb" hastens the excretion of nickel in animals exposed to nickel carbonyl. Further evidence in support of this has been obtained in humans accidentally exposed to nickel carbonyl who have received "Dithiocarb" therapeutically. These clinical studies will be reported in the accompanying paper.

TABLE 6.—THE CONCENTRATION OF GLUCOSE IN THE BLOOD OF RABBITS AFTER THE ADMINISTRATION OF DITHIOCARB

		Blood Glucose mg./100 ml.				
	Dithiocarb Dosage	*Before*	*After Dithiocarb*			
Rabbit	*mg./kg.*	*Dithiocarb*	*2 hrs.*	*24 hrs.*	*48 hrs.*	*96 hrs.*
1	100	120	172	115		
2	100	105	177	157		
3	100	101	132	95		
4	100	88	157	132	112	
5	100	82	140	155	85	
6	100	82	132	97	108	
7	250	92	150	100	100	75
8	250	112	202	135	135	95
9	250	95	147	147	130	97

Summary. The therapeutic effectiveness of dithiocarbamates has been studied in experimental animals receiving lethal inhalations of nickel carbonyl. Of the various derivatives investigated the sodium salts of diethyl, dimethyl, diisopropyl dithiocarbamic acids and sodium morpholine dithocarboxylate appear to be the most effective. Of these 4, sodium diethyldithiocarbamate (Dithiocarb) is the least toxic.

"Dithiocarb" administered intraperitoneally in dosages of 50 or 100 mg. per kg. of body weight provided complete protection against the otherwise lethal effects of nickel carbonyl in concentrations from 0.048 to 0.30 mg. per L. in mice and 0.50 to 1.99 mg. per L. in rats. "Dithiocarb" administered orally also provided moderate protection in a dosage of 100 mg. per kg. of body weight.

Greater amounts of nickel were excreted in the urine and feces of rats exposed to the vapors of nickel carbonyl and treated with "Dithiocarb" than in surviving untreated animals similarly exposed.

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SUMMARIO IN INTERLINGUA

Invenenamento A Nickel. VII. Le Efficacia Therapeutic De Dithiocarbamatos Alcoylate In Animales Experimental Exponite A Nickel Carbonylate

Le efficacia therapeutic de dithiocarbamatos esseva studiate in animales experimental recipiente inhalationes letal de nickel carbonylate. Inter le varie derivatos investigate, le sales natric del acidos diethyl-, dimethyl-, e diisopropyl-dithiocarbamic e morpholino-dithiocarbamato de natrium pare esser le plus efficace. Ex iste 4, diethyldithiocarbamato de natrium (abbreviate como "Dithiocarb") es le minus toxic.

Dithiocarb administrate per via intraperitonee in doses de 50 o 100 mg per kg de peso corporee provideva un protection complete contra le alteremente mortal effectos de nickel carbonylate in concentrationes de inter 0,048 e 0,3 mg per litro in le caso de muses e de inter 0,5 e 1,99 mg per litro in le caso de rattos. Dithiocarb administrate per via oral etiam provideva moderate grados de protection in un dosage de 100 mg per kg de peso corporee.

Plus grande quantitates de nickel esseva excernite in le urina e le feces de rattos exponite a vapores de nickel carbonylate e tractate con Dithiocarb que in le urina e le feces de rattos similemente exponite e supervivente sin tractamento con Dithiocarb.

SUMMARIO IN INTERLINGUA

(See page 1 for original article)

Le Association De Syndrome Carcinoide In Function Con Scleroderma. I. Reporto De Un Caso

Es describite un caso de syndrome carcinoide in function. Durante su vita le patiente exhibiva accessos paroxysmal de rubor cutanee con telangiectasia, dolor colicoide del abdomine e diarrhea, murmures cardiac, e alterationes sclerodermoide del extremitates inferior. Accessos de rubor e edema poteva esser precipitate per le ingestion de bibitas alcoholic o stresses emotional.

Le necropsia revelava carcinoide maligne del ileum con metastases a hepate, pulmon, corde, mamma, e appendice. Alterationes fibrotic esseva observate non solmente in le hepate, le vena cave inferior, e le corde dextere sed etiam in le pelle del gambas e in association con le metastases de tumor. Es discutite le signification que possibilemente inhere in iste observationes.

NICKEL POISONING

VIII. DITHIOCARB*: A NEW THERAPEUTIC AGENT FOR PERSONS EXPOSED TO NICKEL CARBONYL†

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This paper is concerned with clinical observations on persons accidentally exposed to nickel carbonyl and treated with sodium diethyldithiocarbamate (Dithiocarb). It complements the previous paper[6] which presents our studies of the striking effectiveness of Dithiocarb in counteracting the lethal effects of nickel carbonyl in experimental animals.

Nickel carbonyl is one of the most toxic chemicals encountered industrially.‡ Hazards from exposure may arise from a variety of operations. These include 1) the separation of nickel from its ores; 2) the preparation of intermediates in organic syntheses, sometimes under extremely high pressures; 3) electroplating operations as a medium for depositing thin layers of metallic nickel in electronic circuits and magnetic tapes; and 4) inadvertent formation whenever carbon monoxide comes into contact with an active form of nickel. In the latter case, the hazard of exposure is increased because the presence of nickel carbonyl may not be easily recognized. Nickel carbonyl lacks any strong or penetrating odor to warn of its presence. Furthermore, severe symptoms may develop insidiously hours or even days after exposure. In a tragic accident which we reported previously[3], approximately 100 men were exposed to nickel carbonyl of which 31 required hospitalization and 2 died. It is noteworthy that in this accident nickel carbonyl was not recognized as the offending agent until 4 days after exposure.

The boiling point of nickel carbonyl is 43° C. and its vapor pressure at 25° C. is 380 mm. Hg. This high volatility makes it difficult to avoid exposure by inhalation during handling.

EFFECTS OF EXPOSURE TO NICKEL CARBONYL. The symptoms which follow acute exposure to nickel carbonyl are of 2 types, initial and delayed. The initial symptoms are generally mild and may disappear quickly when the subject is brought into uncontaminated air. Initial symptoms include headache,

*"Dithiocarb" is the authors' abbreviation for sodium diethyldithiocarbamate trihydrate.

†This study was supported in part by contract with the Atomic Energy Commission.

‡The nineteenth American Conference of Governmental Hygienists (April, 1957) placed the maximal allowable concentration for an exposure of 8 hours at 0.001 parts per million parts of air (by comparison, hydrogen cyanide was placed at 10 parts per million)[1].

giddiness, nausea, coughing, dyspnea and weakness. Some patients may feel well for several days after the initial symptoms disappear and then develop the more serious delayed reactions. In other patients the 2 stages may merge. Delayed reactions usually commence with severe retrosternal pain and complaints of tightness in the chest. The patients develop cough, dyspnea, weakness and occasionally, gastrointestinal and genitourinary symptoms, and terminally, delirium and convulsions. Detailed description of the clinical course has been reported in a previous paper[3].

Within recent years the high incidence of carcinoma of the lungs and respiratory passages among workers of long service in nickel refineries has come to be recognized (Kincaid *et al.*[2]). The bulk of the evidence would tend to incriminate nickel carbonyl as the cause of the cancer. Our recent studies on the chronic exposure of rats to nickel carbonyl support this hypothesis[4,5].

SODIUM DIETHYLDITHIOCARBAMATE (DITHIOCARB). A summary of the physical and chemical properties of Dithiocarb is given in Table 1. Pharmacologic studies on the effectiveness of this chemical and related compounds are given in the preceding paper. It is noteworthy that aqueous solutions of Dithiocarb are alkaline. Epigastric discomfort following ingestion may be minimized by the simultaneous administration of a buffer, such as sodium bicarbonate. For parenteral administration the chemical is buffered with monosodium phosphate to yield a solution of pH 7.4.

TABLE 1.—PROPERTIES OF SODIUM DIETHYLDITHIOCARBAMATE TRIHYDRATE ("DITHIOCARB")

$(C_2H_5)_2N-\overset{\overset{S}{\|}}{C}-S\ Na\ .\ 3H_2O$ Mol. wt. 225.3

Appearance:	White crystaline solid
Melting Point:	90–92° C.
Solubility:	Soluble in water, methanol, ethanol, and acetone Insoluble in ether and benzene
Stability:	Stable at room temperature Unstable in acid solutions
LD_{50}:	Mice and rats (I.P. and oral)—1.5 gm./kg. body wt.

A 10% aqueous solution of sodium diethyldithiocarbamate trihydrate yields a pH value of 11.6 at room temperature. This solution may be buffered with monosodium phosphate to pH 7.4. At pH concentrations lower than 7.4, the mixture becomes turbid and decomposes, developing an odor of H_2S.

Clinical Studies. During the past 5 years, the senior author participated as a consultant in the medical management of approximately 165 persons accidentally exposed to the vapors of nickel carbonyl. The exposures occurred in conjunction with several different types of industrial operations. Six of the persons were heavily exposed to nickel carbonyl this past year and were treated with Dithiocarb. Five additional workers received 1 or 2 doses of Dithiocarb following mild exposure. The therapy in these subjects was discontinued when urine analyses failed to reveal increased concentrations of

nickel. Thus, to date, 11 persons exposed to nickel carbonyl have received Dithiocarb medication.

The following case summary is illustrative of the patients with severe exposure who were treated with Dithiocarb. Patient D, a healthy, 25-year-old industrial worker, was accidentally sprayed with nickel carbonyl while cleaning a decontamination furnace. He immediately developed dizziness, nausea, hyperpnea, cyanosis, and constricting pain in the chest. The patient was removed from the area and resuscitated by inhalation of a mixture of 95% oxygen and 5% carbon dioxide. After the contaminated clothing had been removed, and the patient had been wrapped in warm blankets, he was given 2 gm. of Dithiocarb orally, along with 2 gm. of sodium bicarbonate. The patient was then hospitalized, and treated with Dithiocarb in an oral dosage of 1 gm. twice daily for 10 days following exposure. The patient became asymptomatic after the second day of hospitalization, and he developed no noteworthy delayed reaction. Routine blood and urine analyses were within normal limits throughout the period of observation.

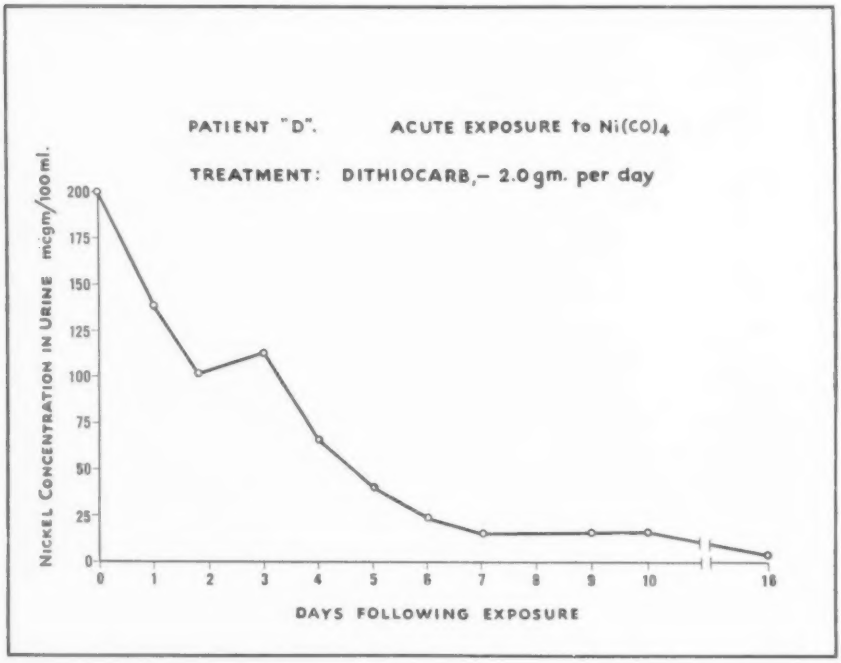

Fig. 1.—Nickel in urine of patient "D" after exposure.

A glucose tolerance test performed on the fourth day after exposure was normal.

On Fig. 1 are presented measurements of the nickel concentration in patient D's urine during the 16 days after exposure. The upper normal limit for the concentration of nickel in urine is 3.0 mcg. per 100 ml. It will be noted that the initial concentration of nickel in this patient's urine was 200

mcg. per 100 ml. This is the highest concentration we have observed in any exposed individual. It will be seen that patient D continued to excrete increased concentrations of nickel in urine throughout the period of Dithiocarb therapy.

In Table 2 are tabulated measurements of nickel concentration in urine obtained from each of the 6 subjects who suffered from heavy exposure and were treated with Dithiocarb. Patients A, B, and C did not receive Dithiocarb until the eighth day after exposure when they began to develop delayed reactions. It is noteworthy that the delayed symptoms were alleviated within a few hours after the administration of Dithiocarb. A significant increase in the concentration of nickel in urine occurred in each of these patients following the ingestion of 1 gm. of Dithiocarb (Table 2). A similar increase in nickel concentration in urine was observed in patient F, in whom Dithiocarb therapy was begun on the fourth day after exposure. By the sixteenth day, the concentrations of nickel in the urine samples from each of the 6 subjects had decreased to less than 5 mcg. per 100 ml. All of the patients made uneventful and complete recoveries, and were able to return to work within 2 to 3 weeks. By way of contrast, in our previous report of 31 cases of severe exposure treated with Dimercaprol[3], 2 patients died, and in the majority of patients convalescence required several months. Furthermore, unpleasant side reactions did not develop after the administration of buffered dithiocarb, as usually develop with Dimercaprol.

TABLE 2.—CONCENTRATION OF NICKEL IN THE URINE OF WORKERS ACCIDENTALLY EXPOSED TO NICKEL CARBONYL AND TREATED WITH DITHIOCARB

Subject	*Time After Exposure (Days)*																
	0	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*
A	28	75	—	41	—	—	—	—	21°	27	—	—	32	—	17	9	2.6
B	—	59	—	—	—	—	—	—	19°	38	33	—	—	2.8	—	—	1.9
C	—	35	—	—	—	—	—	—	24°	34	—	—	18	22	12	—	3.1
D	200°	138	102	117	66	40	23	15	—	15	15	13	9	9	4.4	—	4.4
E	50°	130	87	25	12	25	—	—	32	32	—	—	—	3.9	1.7	2.5	1.4
F	—	62	—	19	9°	20	6	—	3.5	—	—	3.4	—	—	—	—	—

° = Time when Dithiocarb therapy was initiated.

(The nickel concentrations are expressed as mcg. per 100 ml.)

MANAGEMENT OF ACUTE EXPOSURE TO NICKEL CARBONYL. On the basis of our clinical and experimental studies, certain guides are offered for the treatment of persons acutely exposed to the vapors of nickel carbonyl. It is suggested that after exposure the patient be taken immediately away from the contaminated area and the outer clothing removed. If the patient has collapsed, the same resuscitative measures should be employed as are customarily used to treat carbon monoxide poisoning. In addition, an 8-hour collection of urine is begun for nickel analysis.

Measurement of nickel *concentration*° in the initial 8-hour collection of urine has proved to be a valuable aid in classifying patients. If the patient's exposure to nickel carbonyl was a heavy one and typical initial symptoms appear, Dithiocarb therapy may be instituted immediately, either orally or if necessary, by parenteral routes. If there is any doubt regarding the severity of exposure and if the symptoms are minimal, decisions regarding therapy may be deferred until the result of urine analysis for nickel is obtained.

If the initial 8-hour specimen of urine has a nickel concentration of less than 10 mcg. per 100 ml., the exposure may be classified as *mild*. In such cases, it is probable that delayed symptoms will either not develop or will be minimal. Most patients in this group are able to continue at work, although a few may complain of fatigue and require rest. Analyses of the nickel concentration in urine should be made each day, until the concentrations have returned to the normal range. If severe delayed symptoms develop unexpectedly, it is suggested that these patients be hospitalized and given Dithiocarb immediately.

If the concentration of nickel in the first 8-hour collection of urine is above 10 mcg. but less than 50 mcg. per 100 ml., the exposure may be classified as *moderately severe*. Since delayed symptoms frequently develop in these patients, they should remain at rest for at least a week. Dithiocarb may be administered orally in doses of 0.5 gm. 3 or 4 times daily together with 0.5 gm. of sodium bicarbonate and a full glass of water. In our patients Dithiocarb therapy was continued until the patients were free of symptoms and the concentrations of nickel in urine were less than 10 mcg. per 100 ml.

If the concentration of nickel in the first 8-hour collection of urine is above 50 mcg. per 100 ml., the exposure may be classified as *severe*. These patients are apt to be seriously ill and require hospitalization. Most of these patients can be maintained with oral Dithiocarb therapy as outlined for the *moderately severe* group. However, if the patient's condition is critical, it is suggested that Dithiocarb be administered parenterally in an initial dosage of 25 mg. per kg. of body weight and that the total amount during 24 hours be limited to 100 mg. per kg. of body weight.† It should be emphasized that thus far, none of our patients has received Dithiocarb parenterally.

It is suggested that patients receiving Dithiocarb abstain from alcoholic beverages. One of the heavily exposed patients in our series ingested alcoholic beverages 2 days after discontinuing Dithiocarb. The attending physician noted that this patient experienced symptoms similar to those described after Antabuse. The relation-

°Previously reported measurements of the concentration of nickel in the urine of 69 normal persons revealed a mean concentration of 1.1 mcg. per 100 ml. (S.D. ± 0.9)[2]. These data have led to the conclusion that only one specimen in 50 selected from a normal population will be found to exceed a value for nickel concentration in urine of 3.0 mcg. per 100 ml. This value was therefore selected as the upper limit of normal. Measurements of nickel *concentration* in urine have proven more satisfactory than estimations of the urinary *excretion* of nickel, because of the difficulty of obtaining from industrial workers accurate measurements of the volume of urine excreted within stipulated periods of time.

†The solution of Dithiocarb for parenteral injection is prepared by adding 10 ml. of a sterile solution of phosphate buffer (0.5 gm. NaH_2PO_4 per 100 ml.) to 1 gm. of powdered Dithiocarb contained in a sterile ampule. The resulting solution contains 100 mg. of Dithiocarb per ml.

ship between Antabuse and Dithiocarb has been discussed in the preceeding paper[6].

Summary. Studies are reported on 11 persons who received Dithiocarb (sodium diethyldithiocarbamate) after accidental exposure to the vapors of nickel carbonyl.

In the exposed persons the manifestations of nickel carbonyl poisoning were relieved after the ingestion of Dithiocarb. Delayed reactions were minimal and convalescence was uneventful.

On the day after the administration of Dithiocarb, the concentration of nickel in urine became increased in each of the patients subjected to severe exposure. With continued therapy, the concentrations of nickel in urine gradually decreased and reached approximately normal levels within 16 days after exposure.

A plan is proposed for the medical management of patients suffering from the effects of acute exposure to nickel carbonyl.

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SUMMARIO IN INTERLINGUA

Invenenamento A Nickel. VIII. Diethyldithiocarbamato De Natrium: Un Nove Agente Therapeutic Pro Le Tractamento De Personas Exponite A Nickel Carbonylate

Es reportate studios executate in 11 personas qui recipeva diethyldithiocarbamato de natrium (abbreviate per le autor como "Dithiocarb") post haber essite exponite accidentalmente a vapores de nickel carbonylate.

In le personas exponite, le manifestationes del invenenamento per nickel carbonylate esseva alleviate post le ingestion de Dithiocarb. Le reactiones tardive esseva minimal, e le reconvalescentia passava sin incidente digne de nota.

Le die post le administration de Dithiocarb, le concentration de nickel in le urina cresceva in omne le patientes severmente exponite. In le curso del therapia, le concentrationes de nickel in le urina decresceva gradualmente e attingeva valores approximativemente normal intra 16 dies post le exposition.

Es proponite un plano pro le tractamento medical de individuos qui suffre del effectos de exposition acute a nickel carbonylate.

OBSERVATIONS ON AMANOZINE (W-1191-2), A TRIAZINE DIURETIC COMPOUND*

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and

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The search for a potent, nontoxic, orally effective nonmercurial diuretic has led to the evaluation of various derivatives of formoguanamine, which has long been known to be a potent but toxic diuretic. One of these derivatives is a triazine compound, chlorazanil, which has been shown to be a potent oral diuretic but which has demonstrated occasional, moderate elevations of blood urea nitrogen when given in effective doses (Ford *et al.*[1]). Amanozine (W-1191-2) is another triazine compound, an analogue of chlorazanil, which has been offered to further the search for a potent, nontoxic triazine diuretic (Warner-Chilcott[2]). This new compound, amanozine, was investigated to delineate its diuretic potency, its mechanism of action as revealed by its particular electrolyte excretion pattern, and its effectiveness and undesirable side effects, if any, with prolonged and repetitive daily oral administration.

Methods and Materials. Two groups of patients cooperated in the study of this compound. One group consisted of hospitalized, amabulatory male patients with a roughly equivalent controlled cardiac failure. These patients were maintained in an air-conditioned metabolic ward under the observation of trained personnel. Each patient was weighed daily after voiding, before breakfast. All medications required for the control of cardiac failure with the exception of diuretic agents, were continued throughout the study. Their diet contained approximately 300 gm. of carbohydrate, 100 gm. of protein and 100 gm. of fat, with a total of 2800 calories per day. The sodium content of each patient's diet was exactly 50 mEq. per 24 hours. The patients drank 3 liters of distilled water allotted fractionally to assure a constant water intake throughout the day. They were allowed to equilibrate on this diet until each patient excreted a constant amount of sodium which approximated 90% (45 mEq. per 24 hours) of their dietary sodium.

A dose-response curve was determined for amanozine. Ten patients were given the drug orally in doses of 150 mg. and 300 mg. at 6:00 a.m. and 6:00 p.m. on the drug day. Before the administration of each succeeding dose, several days were allowed for the restoration of equilibrium of water and electrolyte (especially sodium) stores. Urine was collected in 24-hour periods and analyzed for sodium and volume before, during, and after each drug period.

The effect of amanozine on the urinary excretion of various electrolytes, and water was observed in 4 patients. Urine was collected under oil in six periods per 24 hours (three 2-hour periods and three 6-hour periods). These specimens were analyzed for sodium, potassium, chloride, phosphate, titratable acidity, ammonia, bicarbonate, total solute and volume. Two of the 4 patients received 150 mg. of amanozine as a single oral dose at 6:00 a.m. and another 150-mg. dose at 6:00 p.m. of the same day. Two other patients received 300 mg. of amanozine as a single oral dose at 6:00 a.m. and another 300 mg. dose at 6:00 p.m. of the same day.

A simple nitrogen balance study was conducted on a single patient. This patient, an intelligent and cooperative white man, was

*Supported in part by a grant from the Houston Heart Association.

maintained on the metabolic ward under the conditions already described. His diet was analyzed for its nitrogen, sodium, chloride, phosphate and potassium content. Discrete renal function studies were obtained before drug therapy and at the end of drug therapy. At the end of a 5-day period of equilibration, the patient was given a carmine marker to delineate the start of the 5-day control period and subsequent stool collections were obtained according to standard techniques analyzed for the same moieties as the diet using 5-day periods. On the last day of each period the patients' hematocrit, blood urea nitrogen, creatinine, inorganic phosphorus, and serum sodium, potassium, chloride and carbon dioxide combining power were determined. On each of the 5 days of the first urinalyses were obtained on each patient before amanozine was given and again at the termination of the 21-day drug period.

Amanozine was given to 6 female outpatients in whom chronic water retention due to unknown cause(s) was present. They were otherwise apparently normal. During the total period of observation, each patient remained on a diet containing 50 mEq. of sodium daily and drank 3 liters of distilled water distributed equally throughout the day. Patients were weighed daily and their daily urinary sodium and urine output was measured. After 3 days on this regimen, each patient received 150 mg. of amanozine as a single oral dose. After a lapse of 4 days (for the re-establishment of equilibrium) 300 mg. of amanozine as a single oral dose was given.

TABLE 1.—THE EFFECT OF ORALLY ADMINISTERED AMANOZINE (W-1191-2) ON SODIUM AND WATER EXCRETION

(Average values for 10 patients at each dose)

Dose (mg.)		*Urine Sodium (mEq./24 hrs.)*			*Urine Volume (L./24 hrs.)*		
		C	*D*	*I*	*C*	*D*	*I*
300		47	64	17	3.00	3.49	0.49
	P value less than			.001			.001
600		48	68	20	2.99	3.48	0.49
	P value less than			.001			.001
	P value between the two doses			N.S.			N.S.

Key: C = Control values (average of two preceding 24-hr. periods)
D = Values on day of drug administration
I = Increase in excretion due to the drug

drug period, the patient received 150 mg. of amanozine orally at 6:00 a.m. and again at 6:00 p.m. of the same day. During the second drug period the patient received 300 mg. of amanozine orally at 6:00 a.m. and again at 6:00 p.m. of the same day for 5 consecutive days. This was followed by a 5-day post-drug period.

The effects of amanozine after chronic daily therapy was investigated in a group of 10 controlled cardiac outpatients. All 10 patients were also hypertensive and were being treated with Rauwolfia and a ganglionic blocking agent. Each patient was continued on the anti-hypertensive medications, and given amanozine, 150 mg. orally twice daily for a period of 21 days. Blood urea nitrogen, endogenous creatinine clearances (two consecutive 30-minute periods), 15-minute PSP excretion rates (after intravenous PSP) and

Results. DOSE-RESPONSE RELATIONSHIPS (Table 1). Amanozine given in an oral dose of 150 mg. twice daily caused an increase in sodium excretion of 17 mEq. and water excretion of 0.49 liters per 24 hours. Following the administration of 300 mg. of amanozine twice daily, there was an increase in sodium excretion of 20 mEq. per 24 hours and an increase in water excretion of 0.49 liter per 24 hours. The "p" values between the 24-hour responses to 300 mg. and 600 mg. of the compound in water and sodium excretion were not significant. The administration of 600 mg. per day (300

mg. twice daily) resulted in gastrointestinal disturbances, namely nausea and vomiting, in the majority of patients receiving this dose. Five of the patients received a dose of 150 mg. once daily but the diuretic effect was not significant.

the same day are presented in Fig. 1. A significant water diuresis was observed, with maximum diuresis occurring 6 to 12 hours after the initial dose. Thereafter a constant decline in diuresis was noted despite the administration of the second dose. The initial

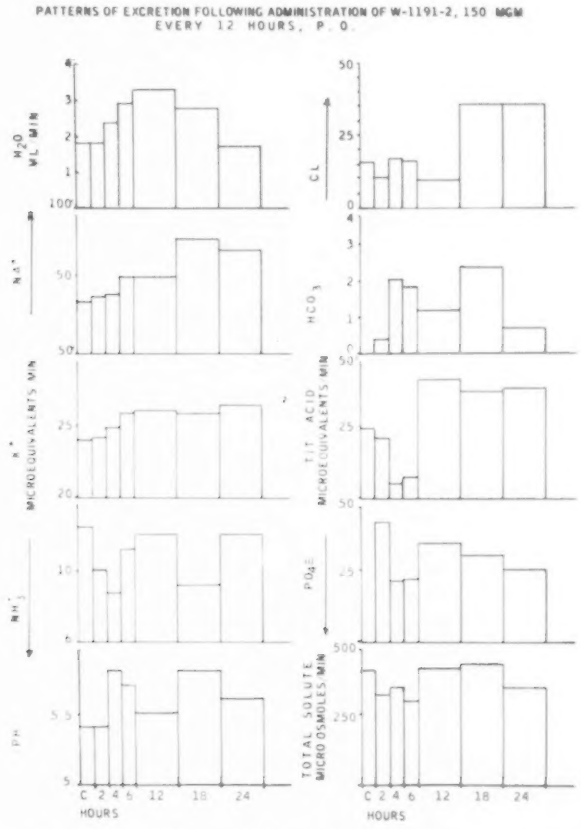


Fig. 1.—Patterns of excretion following administration of oral amanozine (W-1191-2), 150 mg. every 12 hours.

THE EFFECT OF AMANOZINE (W-1191-2) ON THE URINARY EXCRETION OF VARIOUS ELECTROLYTES AND WATER. The average excretion patterns obtained after administration of 150 mg. of amanozine as a single oral dose at 6:00 a.m. and repeated at 6:00 p.m. of

dose of the drug caused a slight increase in the rate of sodium excretion but a much greater effect was seen after the patients received the second dose. Potassium excretion was increased slightly without further effect noted following the 6:00 p.m. dose. The effect

on chloride excretion simulated the sodium excretion pattern, namely a slight increase noted following the initial dose, followed by a marked response after the second dose. Ammonia excretion was profoundly depressed initially, followed by a rapid return to normal levels within 12 hours. This period; the second dose did not significantly alter this increased rate. Bicarbonate increased after each dose but the total quantity of bicarbonate excretion per 24 hours was relatively insignificant. Phosphate excretion fell but the fall was of short duration, with rapid return to control levels. A simi-

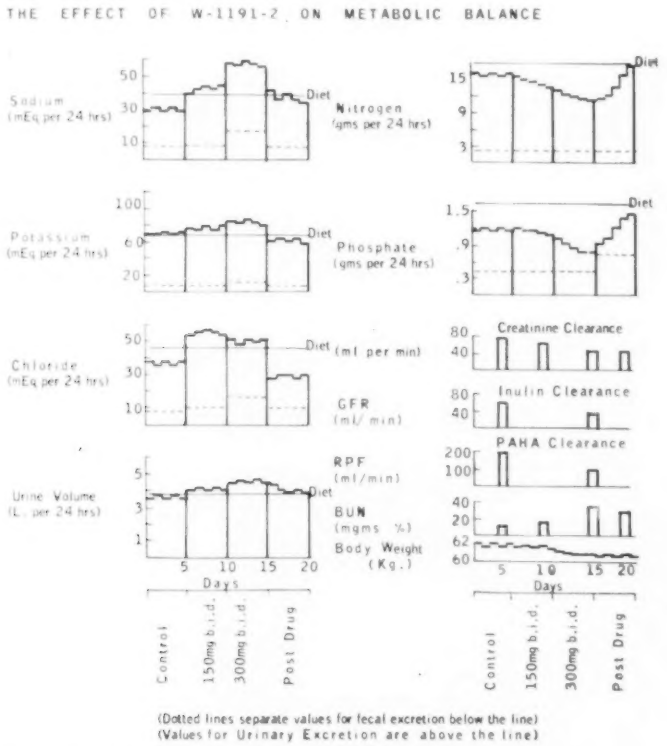


Fig. 2.—The effect of amanozine (W–1191–2) on metabolic balance (one patient).

transitory depression was again demonstrated after the second dose. Urinary pH was rapidly altered towards alkalinity for short periods following each dose of the drug. Conversely, titratable acidity of the urine declined markedly after the initial dose, but the effect was transitory and within 12 hours after the initial dose, the rate of excretion exceeded that noted during the control lar fall was recorded after the second dose.

THE EFFECT OF AMANOZINE (W-1191-2) ON METABOLIC BALANCE (Fig. 2). Amanozine caused more sodium, potassium, chloride and water to be excreted than the daily diet provided. Conversely, nitrogen and phosphate excretion fell below daily dietary intake. During the second drug period

TABLE 2.—THE EFFECT OF CHRONIC ORAL ADMINISTRATION OF AMANOZINE (W–1191–2) ON RENAL DYNAMICS (10 PATIENTS)

(150 mg. Orally Twice Daily for 21 Days, to Hypertensive Patients with Controlled Failure)

	Blood Urea Nitrogen (mg./100 ml.)			*Creatinine Clearance (mg./minute)*		*PSP Excretion % in 15 Minutes*		*Urinalysis (Albumin or Cells)*	
Patient	*C*	*D*	*PD*	*C*	*D*	*C*	*D*	*C*	*D*
1.	13	18	9	76	43	15	10	0	A + C
2.	18	28	20	103	52	13	9	A	A + C
3.	23	30	22	84	71	24	15	A	A
4	27	35	27	133	100	14	9	A	A
5.	24	40	28	132	90	5	3	A	A
6.	18	38	19	89	62	20	10	0	0
7.	12	26	14	99	70	18	10	0	0
8.	10	21	14	100	57	18	9	0	0
9.	13	15	13	77	100	20	21	0	0
10.	22	30	20	109	70	10	3	A	0
Average	18	28	19	100	72	16	10		
P value		<.001	NS		<.001		<.01		

C = Control values
D = Values at the end of 3 weeks' therapy
PD = Values 3 weeks after discontinuation of therapy

TABLE 3.—THE EFFECTS ON BLOOD PRESSURE AND BODY WEIGHT FOLLOWING CHRONIC THERAPY WITH AMANOZINE (W–1191–2)

(150 mg. Orally Twice Daily for 21 Days to Hypertensive Patients*)

BLOOD PRESSURE

	Control				*Drug*				*Post Drug*				*Body Weight (pounds)*		
	Recumbent		*Standing*		*Recumbent*		*Standing*		*Recumbent*		*Standing*				
Patient	*S†*	*D†*	*S*	*D*	*S*	*D*	*S*	*D*	*S*	*D*	*S*	*D*	*C‡*	*D‡*	*PD‡*
1.	200	120	180	110	180	110	150	90	200	120	180	110	160	158	160
2.	220	128	214	120	246	120	216	126	220	130	220	130	156	154	156
3.	200	110	160	90	180	110	140	80	200	110	160	90	148	145	147
4.	180	110	140	70	170	100	120	80	180	110	140	70	151	147	149
5.	160	100	130	80	150	100	110	70	160	100	130	80	165	163	165
6.	184	120	210	128	200	110	124	90	210	120	180	116	174	172	174
7.	200	104	104	58	238	120	152	90	200	120	210	110	135	134	136
8.	176	90	150	100	174	100	150	100	174	94	156	100	184	182	184
9.	228	96	220	90	224	100	194	108	200	100	190	110	136	137	138
10.	200	120	160	110	180	110	140	80	200	120	160	110	132	130	132
Average	195	110	167	96	194	108	150	91	194	112	173	103	154	152	154
‡Mean B.P.	138		119		137		110		139		126				
P value							NS				NS			<.01	NS

All patients continued therapy with previous instituted Rauwolfia plus ganglionic blocking agent except Pt. 8.
F–C = Control Values — D = Drug = Values after three weeks' therapy (W–1191–2) — PD = Post Drug = Values 3 weeks after discontinuation of W–1191–2) therapy.
†S= Systolic—D = Diastolic
‡Mean B.P. = Diastolic Pressure plus 1/3 Pulse Pressure.

when the dose was doubled, the already noted effects became accentuated. Nitrogen retention was correlated with a rising blood urea nitrogen, and decreasing creatinine, inulin and PAHA clearances. Nitrogen and phosphate retention did not persist following the cessation of drug therapy and blood urea nitrogen began to decrease. Body weight was not significantly altered during the period of this study but was slightly decreased.

THE EFFECT OF CHRONIC THERAPY WITH AMANOZINE (W-1191-2) (Tables 2 and 3). Ten patients, when given 150 mg. of amanozine twice daily over a period of 21 days, demonstrated a slight to moderate increase in blood urea nitrogen. Creatinine and PSP clearances fell significantly after 21 days on the drug. Blood urea nitrogen levels returned to normal control levels within 3 weeks after the drug was discontinued. Albumin and casts appeared

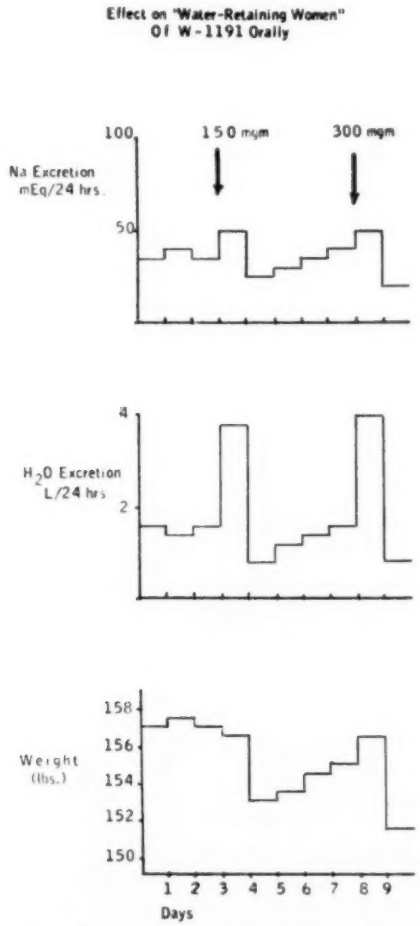
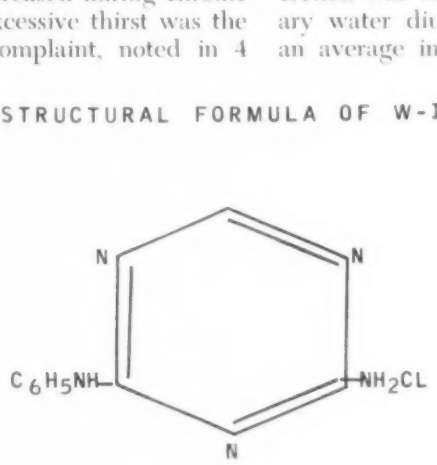


Fig. 3.—The effect of oral amanozine (W-1191-2) on "water-retaining" women.

in the urine of only one patient whose control urine had been normal. Standing blood pressures and body weights were slightly decreased during chronic drug therapy. Excessive thirst was the most common complaint, noted in 4 of the 10 patients. One patient developed gripping abdominal pain and voluntarily discontinued the drug after 18 days of therapy. Abdominal pain ceased when the drug was stopped.

THE EFFECT OF ORALLY ADMINISTERED AMANOZINE (W-1191-2) ON "WATER-RETAINING" WOMEN (Fig. 3). When "water-retaining" women were given 150 mg. of amanozine, a moderate increase in 24-hour urinary sodium excretion was observed. The contemporary water diuresis was marked, with an average increase in urinary excretion of 2 liters within 24 hours after the drug was given. The average weight loss paralleled the increased urinary flow. Similar, although no greater, effects were noted following the administration of 300 mg. of this compound.

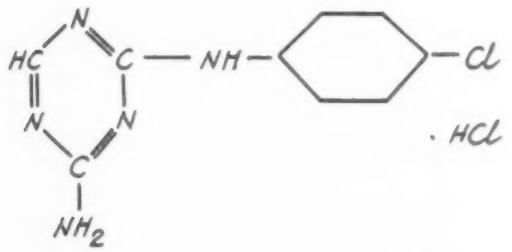
STRUCTURAL FORMULA OF W-1191-2



2-AMINO-4-ANILINO-1,3,5-TRIAZINE HYDROCHLORIDE

Fig. 4.—Structural formula of amanozine (W-1191-2).

STRUCTURAL FORMULA



Chlorazanil hydrochloride

Fig. 5.—Structural formula of chlorazanil (Daquin).

Discussion. The compound of the

present report is a derivative of formoguanamine designated as amanozine (W–1191–2). Chemically, it is 2-amino-4-anilino-1,3,5-triazine hydrochloride, an analogue of another triazine (chlorazanil) whose diuretic properties following oral administration have been reported elsewhere (Ford *et al.*[1]). This new triazine compound has come to our attention because of the problem

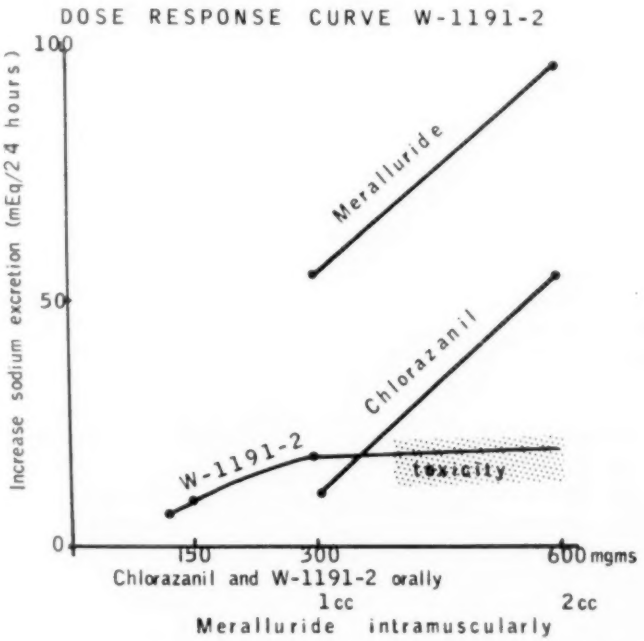


Fig. 6.—Dose-response curve of amanozine (W–1191–2) as compared with meralluride (Mercuhydrin) I.M. and chlorazanil (Daquin) orally.

which was presented with the investigation of chlorazanil, an effective nonmercurial oral diuretic which was found to cause a slight to moderate increase in blood urea nitrogen. The structural formulas for amanozine and chlorazanil are shown in Figs. 4 and 5.

The daily oral diuretic dose of amanozine (W–1191–2) in humans is between 150 and 300 mg. per 24 hours. Doses of 600 mg. per day produced no additional increase in sodium or water excretion and caused severe gastrointestinal disturbances in nearly every case. Its onset of action is noted within 2 hours after oral administration and its duration of action is between 12 to 18 hours. This suggests that a twice daily dose schedule would be maximally effective. In humans, the effect of amanozine on sodium excretion is slight; it is less than one-third as potent as 1 ml. of meralluride (Mercuhydrin) given intramuscularly, and less potent than chlorazanil orally (Fig. 6). Amanozine is, however, a potent "water" diuretic (Table 4). When potency is estimated on the basis of water excretion, 300 mg. of this substance caused a greater degree of water excretion than was noted following 600 mg. of oral chlorazanil or

2 ml. of meralluride (Mercuhydrin) given intramuscularly.* The drug remained moderately effective when given daily. When used in combination with Rauwolfia and ganglionic blocking agents in the treatment of hypertension, it augmented the orthostatic hypotensive effect. Clinical and laboratory evidence of significant but reversible changes in renal hemodynamics and nitrogen balance were detected after 2 to 3 weeks of chronic therapy. Thus, its use in clinical medicine is suggested as a short-term diuretic or perhaps on an interrupted schedule.

The mechanism of action of amanozine (W–1191–2) is reflected in the electrolyte excretion pattern. Thus, augmented water excretion is associated with increased sodium, chloride and bicarbonate excretion. Ammonia and titratable acidity are depressed in reverse relationship to the forenamed. This pattern roughly simulates that noted to characterize chlorazanil and suggests, as with the former drug, the action of yet another diuretic mechanism different from mercurials, carbonic anhydrase inhibitors and chlorothiazide (Diuril).

Conclusions. Amanozine (W–1191–2) is an orally administered nonmercurial triazine diuretic which behaves as a potent "water diuretic" but which causes only a slight augmentation of sodium excretion.

Following chronic oral administration, it is associated with laboratory evidence of significant but reversible depression of renal hemodynamics contemporary with nitrogen and phosphate retention equal to that noted to ensue following chronic therapy with its analogue, chlorazanil. Its use in clinical medicine demands caution, and suggests its usefulness as a short-term oral diuretic.

The mechanism of action of amanozine (W–1191–2) as reflected in its electrolyte excretion pattern simulates that which characterizes its analogue, chlorazanil. Both compounds differ in their mechanism of action from organomercurials, carbonic anhydrase inhibitors and chlorothiazide, suggesting yet a fourth mode of diuretic behavior.

TABLE 4.—COMPARATIVE POTENCY OF AMANOZINE (W–1191–2), CHLORAZANIL (DAQUIN), AND MERALLURIDE (MERCUHYDRIN) AS "WATER DIURETICS"

	W–1191–2 (oral)		*Chlorazanil (oral)*		*Meralluride (intramuscular)*	
	150 mg.	*300 mg.*	*300 mg.*	*600 mg.*	*1 ml.*	*2 ml.*
Increase (L./24 hrs.)	0.15	0.49	0.14	0.39	0.14	0.29

*This effect was reiterated in the marked water diuresis which was noted when the drug was given to "water-retaining" women.

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(Interlingua on page 100)

CAUSE OF DEATH IN PERSONS WITH CONGENITAL HEART DISEASE

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The relatively recent advances in cardiac surgery for congenital cardiac defects make it desirable to estimate the prognosis of such cases as might be considered for surgical treatment.

All necropsied cases with congenital cardiac defects that had survived until 2 years of age or older were included in this series. A total of 84 such cases were found amongst 13,883 necropsies performed over a consecutive 9-year period at the Los Angeles County Hospital.

The majority of the necropsied cases with congenital heart disease not surviving until 2 years of age were stillborn, or were infants that died within a few days of birth. Most of the latter were born prematurely. It was difficult to ascertain the degree to which the cardiac defects contributed to these deaths. Their inclusion would then obviously lower the mean life expectancy of the entire group.

ISOLATED INTERATRIAL SEPTAL DEFECTS. Of the 84 cases with congenital heart disease 58 had an isolated interatrial septal defect. Twenty of these had a defect which measured 0.5 cm. or more in diameter, while in 8 instances the defect was of a smaller size. In the remaining 30 necropsies the size of the defect was not given, but in the majority of these it was stated that the lesion consisted of a foramen ovale which was probably functionally closed during life.

The mean age at death in those patients with an interatrial septal defect over 0.5 cm. in diameter was 53.3 years. Table 1 summarizes the causes of death in this group. Five of the 20 had an associated mitral stenosis, and were representative, therefore, of Lutembacher's syndrome. Three of these died of cardiac decompensation at the ages of 41, 54, and 65 years. The remaining 2 patients with Lutembacher's syndrome died at age 40 and 56 of Laennec's cirrhosis and adenocarci-

TABLE 1.—CAUSES OF DEATH IN 20 CASES WITH AN ISOLATED INTERATRIAL SEPTAL DEFECT WHICH MEASURED OVER 0.5 CM. IN DIAMETER

Age	*Cause of Death*
29	Cardiac decompensation
63	Glioblastoma, right frontal lobe
78	Carcinoma of colon with perforation
80	Old myocardial infarction, coronary atherosclerosis
68	Acute myocardial infarction
70	Embolism to left internal carotid artery
56	Acute myocardial infarction
°43	Ruptured appendix with peritonitis
54	Adhesive pericarditis, acute rheumatic pericarditis
°65	Hypertensive heart disease with cardiac decompensation
2	Staphylococcus septicemia
65	Cardiac decompensation
30	Pulmonary tuberculosis
34	Bronchiectasis with lung and brain abscesses
°40	Laennec's cirrhosis
°56	Adenocarcinoma of ovary with metastasis
63	Hypertensive heart disease with cardiac decompensation
58	Constrictive pericarditis; cardiac decompensation
°41	Cardiac decompensation

°Cases with associated mitral stenosis

noma of the ovary with metastases, respectively.

Among the 15 other cases in this group, 8 died of cardiac causes. One of these died of cardiac decompensation at the age of 29. The other 7 died of a type of heart disease apparently unrelated to the atrial septal defect.

The causes of death of the 8 patients that had an interatrial septal defect under 0.5 cm. in diameter are summarized in Table 2. These causes could not be directly attributed to the defect in any instance.

TABLE 2.—CAUSES OF DEATH IN 8 CASES WITH AN ISOLATED INTERATRIAL DEFECT THAT MEASURED LESS THAN 0.5 CM. IN DIAMETER

Age	*Cause of Death*
73	Uremia, acute glomerular nephritis
69	Carcinoma of stomach with metastases
68	Carcinoma of stomach with metastases
79	Acute myocardial infarction
64	Acute myocardial infarction
40	Bronchial asthma, cor pulmonale
50	Multiple platelet thrombosis
77	Acute myocardial infarction

The 30 necropsies in which no measurement of the atrial septal defect was given had a mean age at death of 55.7 years. The causes of death for this group are summarized in Tables 3A and 3B. It can be seen that these are varied, and cannot be attributed directly to the congenital defect, except possibly in the one patient who died of cardiac decompensation at the age of 52. The 2 instances of brain abscess can scarcely be related to paradoxical embolism through the atrial septal defect since in both instances the pathologist considered that the defects had probably been functionally closed during life.

ISOLATED INTERVENTRICULAR SEPTAL DEFECTS AND EISENMENGER'S COMPLEX. Eleven of the 84 cases with congenital cardiac lesions had an isolated interventricular septal defect. Two had some dextroposition of the aorta, and thus represented Eisenmenger's complex. The mean age at death was 32.1 years. Two patients died at a relatively

TABLE 3A.—CAUSES OF DEATH IN 30 CASES OF ISOLATED INTERATRIAL SEPTAL DEFECT THE SIZE OF WHICH WAS NOT GIVEN BY THE NECROPSY SURGEON

Age	*Cause of Death*
59	Staphylococcus septicemia
60	Cerebral thrombosis
72	Carcinoma of lung with metastases
47	Subdural hematoma
40	Pulmonary tuberculosis
62	Acute myocardial infarction
41	Lung abscess, bronchiectasis
69	Ruptured appendix; portal thrombophlebitis
73	Carcinoma of urinary bladder
75	Acute myocardial infarction
67	Acute myocardial infarction
52	Cardiac decompensation
34	Staphylococcus septicemia
67	Rheumatic mitral stenosis with cardiac failure

TABLE 3B.—CAUSES OF DEATH IN 30 CASES OF ISOLATED INTERATRIAL SEPTAL DEFECT THE SIZE OF WHICH WAS NOT GIVEN BY THE NECROPSY SURGEON

Age	*Cause of Death*
20	Brain abscess
50	Laennec's cirrhosis
11	Brain abscess
69	Bronchopneumonia
68	Bronchial asthma, cor pulmonale
57	Laennec's cirrhosis
71	Uremia, obstructive uropathy
82	*E. coli* septicemia
2	Pneumococcus meningitis
48	Status epilepticus
39	Cerebral thrombosis, luetic (?)
75	Luetic aortic insufficiency, cardiac decompensation
78	Old myocardial infarction; aortic stenosis
72	Silicosis; cor pulmonale
60	Luetic aortic insufficiency with cardiac decompensation

young age of noncardiac causes. Both of these were Mongolian idiots, in whom ventricular septal defects are not uncommon.

Four of this group with isolated interventricular septal defects died with a brain abscess. This may have resulted from the lack of blood filtration by the pulmonary capillary bed (Maronde[2]). The main blood shunt which exists in persons with an isolated interventricular septal defect is from the left to the right ventricle, but a smaller shunt in the reverse direction is also present (Handelsman *et al.*[1]). The 2 patients with Eisenmenger's complex died with severe pulmonary atherosclerosis and cardiac decompensation. Pulmonary hypertension is important in the development of atherosclerosis of the pulmonary arteries. Closure of the ventricular septal defect may decrease

TABLE 4.—CAUSES OF DEATH IN 11 PATIENTS WITH INTERVENTRICULAR SEPTAL DEFECTS

Age	*Cause of Death*	*Comment*
2	Bronchopneumonia	Mongolian idiot
19	Brain abscess	
18	Brain abscess	
37	Cardiac decompensation	Dextroposition of aorta
53	Staphylococcus septicemia	
29	Brain abscess	
55	Sarcoma of uterus	
56	Brain abscess	
7	Scarlet fever	Mongolian idiot
47	Cardiac decompensation	Dextroposition of aorta
31	Cerebral embolism	

TABLE 5.—CAUSES OF DEATH IN 16 CASES WITH COMBINED CONGENITAL CARDIAC DEFECTS OR PATENT DUCTUS ARTERIOSUS

Age	*Cause of Death*	*Cardiac Defects*
5	Brain abscess	Tetralogy of Fallot
6	Subacute bacterial endocarditis	Tetralogy of Fallot
6	Brain abscess	Tetralogy of Fallot
26	Brain abscess	Tetralogy of Fallot
3	Brain abscess	Tetralogy of Fallot
26	Cardiac decompensation	I.A. and I.V. septal defects. Rheumatic panvalvulitis
30	Pneumococcal meningitis	I.A. and I.V. septal defects
16	Brain abscess	Single atrium, I.V. septal defect
13	Brain abscess	Single atrium, I.V. septal defect
7	Subarachnoid hemorrhage	Transposition of great vessels and I.V. septal defect
16	Thrombocytopenic purpura	I.A. septal defect, pulmonary stenosis
14	Subacute bacterial endocarditis	Single atrium, rudimentary right ventricle, I.V. septal defect
6	Brain abscess	Single atrium, rudimentary right ventricle, I.V. septal defect
9	Glomerular nephritis	Patent ductus arteriosus
15	Rheumatic pancarditis	Patent ductus arteriosus
25	Dissecting thoracic aneurysm	Patent ductus arteriosus

the blood shunt but the decrease in the pulmonary hypertension may not be marked if considerable pulmonary vascular resistance has developed prior to the operation.

PATENT DUCTUS ARTERIOSUS AND COMBINED CONGENITAL CARDIAC DEFECTS. The remaining 16 cases with congenital cardiac lesions are summarized in Table 5. Five represented the tetralogy of Fallot, and a brain abscess was the cause of death in 4. These have been reported previously (Maronde[2]).

Three of this group had a patent ductus arteriosus. The causes of death were not cardiac in origin. No conclusions can be reached from this small number of cases. The remaining 8 cases had various combinations of congenital cardiac defects. They died at a relatively young age, some of brain abscesses. Several had defects which might have been amenable to correction or improvement by present-day surgical techniques.

Summary. 1. The causes of death of 84 patients with congenital cardiac defects who had survived until 2 years of age or longer are reported.

2. Patients with isolated interatrial septal defects more often than not die of an unrelated cause, even when the defect is large. Future evaluation of any surgical correction of this defect, unless cardiac symptoms are present, would be difficult.

3. The patients with an isolated interventricular septal defect but without dextroposition of the aorta, had a cause of death unrelated to the defect, except for brain absecesses.

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SUMMARIO IN INTERLINGUA

Le Causa Del Morte In Personas Con Congenite Morbo Cardiac

1. Es reportate le causas del morte de 84 patientes con congenite morbo cardiac qui superviveva usque al etate de 2 annos o plus.

2. Patientes con isolate defectos de septo interatrial mori in un majoritate del casos ab un causa non-relationate, mesmo si le defecto es grande. Le evalutation futur del correction chirurgic de iste defecto, excepte in le presentia de symptomas cardiac, non pote esser facile.

3. Le patientes con isolate defecto de septo interventricular sed sin dexteroposition del aorta habeva causas de morte sin relation a ille defecto, excepte in casos con abscessos cerebral.

AN EXPLANATION FOR THE CENTROLOBULAR LOCALIZATION OF INTRAHEPATIC BILE STASIS IN ACUTE LIVER DISEASES

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Pathologists have long known that obstruction of the extrahepatic bile ducts is reflected in the liver lobules by intrahepatic bile stasis, which is first seen around the central veins and then spreads from the center to the periphery of the liver lobules in a cumulative manner. Furthermore, centrolobular bile stasis is often seen in the acute stage of a wide variety of hepatocellular diseases characterized by liver cell failure in the absence of mechanical obstruction to the flow of bile. A partial list of such diseases includes such diverse conditions as carbon tetrachloride poisoning, leptospirosis, viral hepatitis, and the acute hepatic necrosis of alcoholism. Admittedly, the degree of intrahepatic bile stasis in hepatocellular diseases seldom reaches that seen in complete obstructive jaundice. This quantitative difference does not, however, detract from the striking fact that such completely different conditions as obstructive and nonobstructive liver diseases both demonstrate this centrolobular localization of bile plugs. The ubiquity of this phenomenon implies that the explanation lies in the basic mechanisms of bile flow. The identification of these mechanisms concerns both the physiologist and pathologist. The fact that intrahepatic bile stasis is seen histologically in both obstructive and nonobstructive jaundice, creates serious difficulties for the pathologist who must distinguish extrahepatic biliary obstruction from intrinsic cholestatic ("cholangiolitic") hepatitis on the basis of liver biopsy specimens (Dubin[1]). Moreover, this commonage between obstructive and hepatocellular diseases has led to the assumption that "obstruction" is the basic mechanism at work in the various types of cholestatic hepatitis. For example, the hepatitides caused by such agents as chlorpromazine, organic arsenicals and methyl testosterone (to name but a few) have been blamed upon "obstruction" of the intrahepatic biliary system (Gutman[6]). This viewpoint assumes that, because liver cell necrosis is not prominent in such conditions, the intrahepatic bile stasis must result from intrahepatic obstruction.

We felt that an examination of the dynamics of intrahepatic bile flow would perhaps illuminate this dark and fusty corner of liver pathology. Our consideration of the problem has led us to a working hypothesis which explains the centrolobular localization of intrahepatic bile stasis in the acute stage of both obstructive and nonobstructive liver diseases. This hypothesis is based upon two assumptions, both of which are generally accepted.

The first is that bile flows centrifugally, that is, away from the center and toward the periphery of the liver lobule (Elias[4], Grafflin and Bagley[5],

Popper and Schaffner[9], Rich[10]). The second assumption is that the liver cells normally tend to maintain a constant output of bile (Starling[11]). From these two assumptions it is possible to draw certain conclusions regarding the dynamics of bile flow within the liver lobule. The essentials of our assumptions are presented schematically in Figs. 1 and 2. Figure 1 represents the centrifugal flow of bile, while Fig. 2 shows a model of a group of liver cells

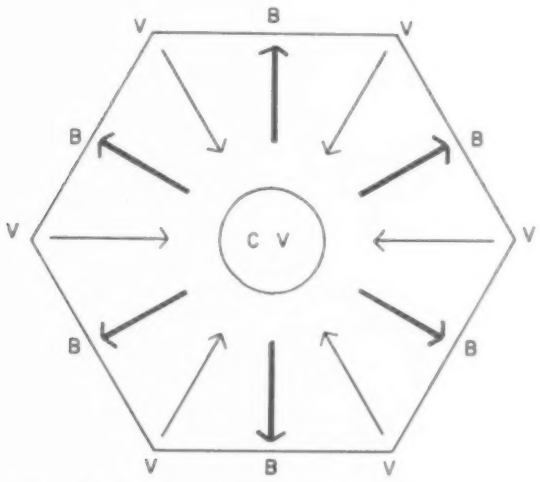

Fig. 1.—Diagram of liver lobule, showing centrifugal flow of bile (from the center to the periphery of the lobule) and the centripetal flow of blood.
CV = central vein; V = vascular channels in portal area; B = bile duct in portal area. The heavy arrows show the direction of bile flow and the slender arrows of vascular flow.

lining a biliary canaliculus into which they elaborate bile. These figures are not intended to portray the precise anatomy of the liver lobule, but only those aspects of liver structure and function that are germane to our argument, namely (a) that bile flow is centrifugal, and (b) that the bile-producing liver cells are arranged sequentially along the biliary canaliculus from the center to the periphery of the lobule.

Using the model presented in Figs. 1 and 2, and applying accepted principles of fluid dynamics (Peterson[8]), one can list the following important features of the dynamics of bile flow within the liver lobule.

A. BILE FLOW. Flow will increase along the length of the channel, *l*, from l_a to l_b. The rate at which it increases will be a function of the number of cells per unit of channel length and the flow of bile from each cell into the channel. For example, in the schema shown in Fig. 2 the flow at l_b would be 10 times that at the point where cells 1 and 2 introduce bile into the channel, provided each cell elaborates the same amount of bile per unit of time.

B. VELOCITY OF BILE FLOW. The velocity of the flow at any site along the channel is a function of the flow rate and the radius of the channel at that site. The radius

is a function of the stiffness* of the channel wall and its surround and also the pressure difference between the inside and the outside of the channel.

prevent motion. All of the forces tending to prevent such a motion are collectively referred to as "total fluid mechanical load." The forces which must be overcome by the

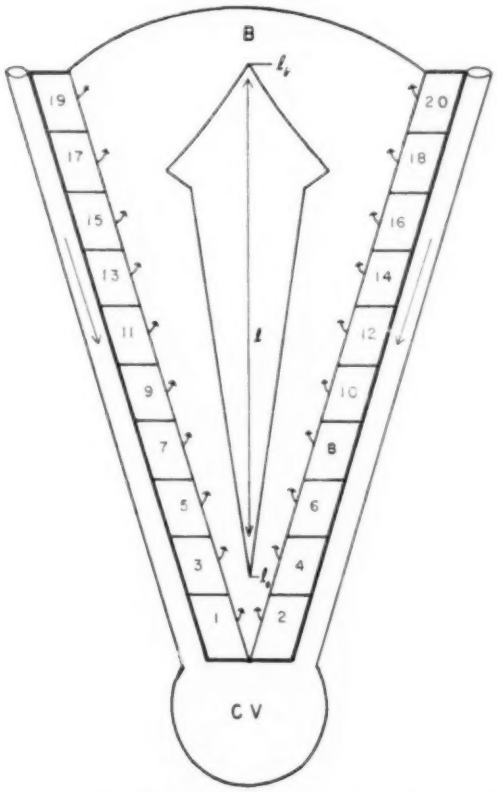

Fig. 2.—Schematic drawing of simplified model of a biliary canaliculus of length, l, surrounded by 20 liver cells (an arbitrary number) which elaborate bile into the channel. The flow of bile is centrifugal, that is, from the center of the lobule (situated at the central vein, CV) to the interlobular bile duct, B, located at the periphery of the lobule. At the entrance to B (l_b) 20 liver cells contribute to the total flow of bile, while at the origin of the canaliculus (l_o) only 2 cells contribute to the flow at that site. The large arrow represents the amount and direction of bile flow.

C. BILE CHANNEL LOAD. Motion of bile, like motion of any other substance, depends upon the balance between the forces tending to produce and those tending to

liver cells to produce any given flow of bile may be lumped into 3 general groups, which we will designate as "factors 1, 2 and 3," respectively.

*The physical properties of a tube are determined by (a) the modulus of elasticity and thickness of both the tube wall and its surround, and (b) the tube radius. Collectively, these factors are called the "stiffness" of the structure (Peterson[8]).

FACTOR 1. Force is required to maintain an open passage, that is, a patent channel. Conversely, then, part of the total load is due to the weight and tension of the tissues surrounding the channel lumen. Little or nothing is known of the magnitude of this particular component of the total load; nevertheless, it is undoubtedly significant.

FACTOR 2. All liquids resist shear (one layer of liquid sliding over another, that is, flow). This resistance is due to fluid friction (viscosity). The magnitude of this resistance to flow is a function of the velocity of the flow, the viscosity of the liquid and the dimensions of the flow channel.

FACTOR 3. The third component of the total load will consist of the force applied in the direction opposite to that of the flow, in this case, the pressure level at the orifice of the distal end of the channel (that is, in the interlobular bile duct).

Consider next how these forces are distributed along the length, *l*, of the channel. The forces tending to close the channel (factor 1) are present along the entire length of the channel. Although we do not know the magnitude and distribution of these forces per unit length, it may be safely assumed that the load derived from this group of forces at any given point will be a function of the length of the channel *beyond* that point. Therefore, the magnitude of this particular load (factor 1) will be greatest at the origin of the channel (located at the center of the liver lobule) and will gradually decrease toward the distal end (periphery of the lobule).

As regards the distribution of factor 2 along the length of the biliary canaliculus, we can restrict ourselves to a consideration of the total viscous effects. The total viscous resistance to be overcome at any given point (other factors being constant) will be a function of the length of the flow path *beyond* that point. That is to say, factor 2 of the total load (like factor 1) will be greatest at the center of the liver lobule and least at the periphery of the lobule.

On the other hand, as regards factor 3, the force applied at the distal end of the biliary canaliculus (represented by the pressure in the interlobular bile duct) will tend to contribute the same load along the entire length of the channel, that is, factor 3 will tend to be identical all along the biliary canaliculus.

The sum of factors 1 and 2 can be considered as the "intralobular component of the total load." This will be greatest at the center of the liver lobule (see Fig. 3). In other words, the cells having to force bile the longest distance must provide the greatest force. Factor 3 can be considered as the "extralobular component of the total load" and this will have the same value all along the length of the biliary canaliculus. However, the total load (comprised of the sum of factors 1, 2 and 3) will still be greatest at the center and least at the periphery of the liver lobule. The cells at the center of the lobule will have to do the most work to propel the bile along the biliary tree (see Fig. 3).

The cardiovascular system provides a useful analogy of the situation in the biliary system. The load of the entire vascular system is referred back to the heart, that is, the heart must provide the energy which is dissipated over the entire flow path. If other hearts were distributed along the system the central heart would still be required to do a relatively greater

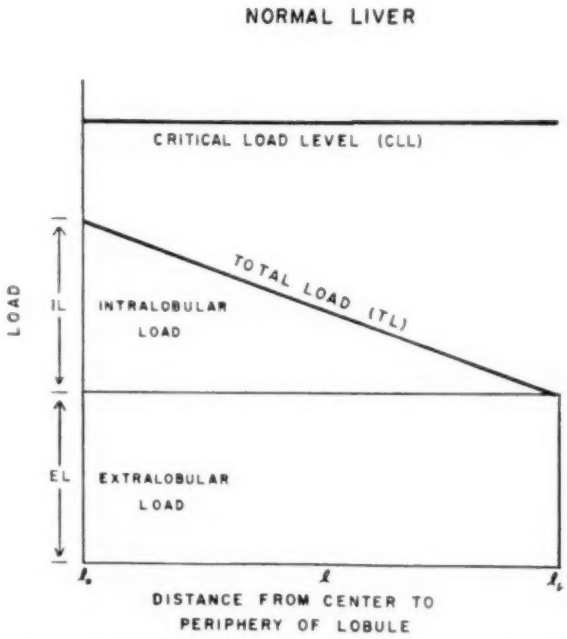

Fig. 3.—Diagram of curve of total fluid mechanical load on liver cells in a normal liver lobule, in relation to the distance (l) between the center (l_a) and periphery (l_b) of the lobule.

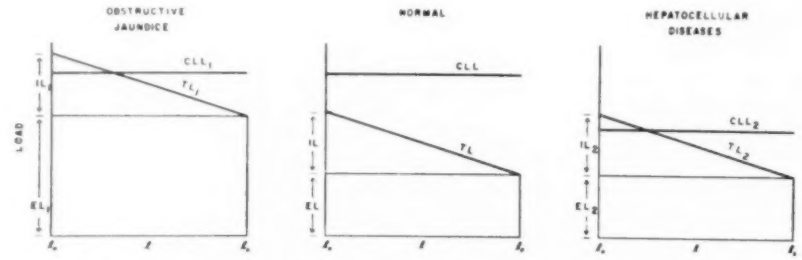

Fig. 4.—Comparison of conditions in the normal liver, obstructive jaundice and hepatocellular diseases, with regard to the critical load level of the liver cells and the total load upon these cells. l = distance from the center (l_a) to the periphery (l_b) of the liver lobule.

In obstructive jaundice the intralobular load (IL_1) and the critical load level (CLL_1) remain relatively normal, but there is a sharp increase in the extralobular load (EL_1). The latter causes an increase in the total load (TL_1), but the slope of the curve of total load remains the same. Consequently, when the total load exceeds the critical load level this occurs first at the center of the lobule.

In hepatocellular diseases the total load (TL_2) remains relatively normal in magnitude and slope of curve. However, the critical load level is sharply reduced (CLL_2), that is, the ability of the damaged liver cells to put out bile is greatly decreased. When the diminished critical load level drops below that of the total load, this occurs first at the center of the lobule.

amount of work to provide a similar flow.

It has been found that the pressure in the extrahepatic bile ducts is normally about 10 cm. of H_2O and that this pressure may be increased to 30 cm. of H_2O before bile flow ceases (Ivy and Goldman[7], Starling[11]). These observations suggest that the liver cells possess the property of responding to an increased load with an increased energy output until some "critical load level" is reached (see Fig. 3). This is not an unique biological function. A well known property of the heart is that it responds to an increased load by producing more energy, (that is, doing more work), thus tending to maintain a constant cardiac output. Many secretory glands are known to behave likewise.

We may now examine what happens under abnormal conditions (see Fig. 4).

OBSTRUCTIVE JAUNDICE. In obstructive jaundice there is obstruction to the flow of bile so that the extralobular bile pressure is increased. This means that the extralobular component of the load on the liver cells will be increased, but this increase will tend to be of the same magnitude along the entire length of the channel. If we refer to Fig. 4, this would be equivalent to raising the height of the ordinate EL along the entire length of the abscissa, *l*, to produce an increased EL_1. The curve of the intralobular component of the total load (IL) may be somewhat modified (IL_1) by the increased extralobular load, but the differential of IL_1 along the lobule will still be maintained, so that IL_1 will tend to remain greatest at the center and least at the periphery of the lobule. The over-all effect of obstructive jaundice on the curve of total load in the lobule will be to raise the height of the curve, but the general slope of the curve will tend to remain the same, so that the new total load (TL_1) will still be greatest in the center and least in the periphery of the lobule. The critical load level in the acute phase of obstructive jaundice can be assumed to remain relatively normal (CLL_1), although evidence suggests that the liver cells are damaged to some degree and the CLL_1 would therefore probably be somewhat diminished. When the new total load (TL_1) exceeds the critical load level (CLL_1) of the liver cells, this will occur first in the center of the liver lobule. Thus, the first place where the liver cells reach their limit (critical load level) and stop secreting bile is in the center of the lobule. This accounts for the centrolobular bile stasis in the earliest phase of obstructive jaundice. As obstructive jaundice persists for a few weeks, more and more liver cells in the lobule reach their limit and fail; this cumulative effect spreads from the center to the periphery of the lobule, and is paralleled by a cumulative increase in intralobular bile stasis from center to periphery.*

HEPATOCELLULAR DISEASES. In hepatocellular diseases the liver cells are damaged, so that there is an impairment of their ability to perform the work necessary to secrete bile. In other words, the critical load level of the liver cells is decreased (see Fig. 4). In a diffuse hepatocellular disease like acute nonfatal viral hepatitis we can assume that the liver cells are damaged equally everywhere in the lobule and that the critical load level will therefore be diminished at an equal

*In chronic biliary obstruction the situation is more complex. Perhaps anastamosing channels open up. Also, the liver cells may adapt themselves to the new conditions and put out bile under a greater load. Histologically, in such conditions there is no special zonal distribution of intrahepatic bile plugs.

rate all along the lobule (CLL_2). Although the intralobular and extralobular components of the total load will be somewhat altered, they will remain relatively normal. Therefore, the total load (TL_2) and the slope of its curve will also remain relatively normal, that is, the total load will still be greatest at the center and least at the periphery of the lobule. Consequently, although the critical load level is diminished equally throughout the lobule, when this diminished critical load level (CLL_2) falls below the level of the total load (TL_2), this will occur first in the center of the lobule. In other words, in hepatocellular diseases, the failure of liver cells to put out bile occurs first in the center of the liver lobule. This in turn is reflected in centrolobular bile stasis, which is what is seen histologically. Thus, even in the absence of intrahepatic obstruction, certain hepatocellular diseases are marked by centrolobular bile stasis merely on the basis of the differential in total load within the liver lobule.*

Thus, whether the load is increased (as in obstructive jaundice) or the critical load level of the liver cells is diminished (impairment of liver cells in hepatocellular diseases), failure of bile secretion occurs first in the centrolobular zone and this is manifested by intrahepatic centrolobular bile stasis. Intrahepatic bile stasis is more severe in obstructive jaundice presumably because the outlets are blocked. In hepatocellular diseases the outlets still remain open and some bile flow occurs from liver cells that are still functioning; furthermore, because liver plates are disrupted, biliary canaliculi are torn open and bile can escape into blood or lymph.

When we examine the problem of the pathogenesis of bile stasis in cholestatic ("cholangiolitic") hepatitis in the light of the foregoing discussion, it appears altogether likely that the various forms of cholestatic hepatitis are not necessarily caused by intrahepatic obstruction but may well be the result of liver cell failure. Failure of the liver cell as a secretory organ can occur without marked necrosis. Indeed selective impairment of the secretory mechanism is pin-pointed in chronic idiopathic jaundice, a familial defect with no hepatocellular necrosis whatsoever (Dubin[3]). Furthermore, not all functions of the liver cell need be simultaneously or uniformly impaired in any given hepatocellular disease. Again, the combination of impairments of specific hepatocellular functions may well differ from one type of hepatocellular disease to another, and these different combinations of malfunctions undoubtedly are reflected in different structural alterations within the cell. Some of these structural changes will be gross enough to be visible with ordinary light microscopy while others would require electron microscopy to demonstrate them. It is conceivable, therefore, that in some forms of acquired liver cell damage there may be only mild necrosis but sufficient impairment of the secretory mechanism to produce the histologic and clinical picture of cholestatic hepatitis. And this can occur without the existence of intrahepatic biliary obstruction.

Summary. This paper presents an

*Intralobular bile stasis does not occur in all acute hepatocellular diseases. Obviously, if the liver cell has completely lost its ability to remove bilirubin from the serum, no bile will be secreted at all. Perhaps this is what happens in yellow fever. Also, intracanalicular bile plugs can be lost by reflux into blood and lymph by way of holes created in canaliculi by disruption of liver plates. The general problem of the pathogenetic mechanisms of jaundice and intrahepatic bile stasis will be treated elsewhere (Dubin[2]).

hypothesis to explain the centrolobular localization of intrahepatic bile stasis in the acute phase of both obstructive and hepatocellular liver diseases. This hypothesis is based upon a consideration of the dynamics of bile flow within the liver lobule. Our considerations indicate that (1) the total fluid mechanical load upon the liver cells tends to be greatest at the center and least at the periphery of the liver lobule, and (2) the liver cells can respond to an increased load with an increased energy output that tends to maintain bile flow, until some "critical load level" is reached. Failure of bile secretion is brought about when the total load upon the liver cells exceeds their critical load level. This failure occurs in obstructive jaundice because the total load upon the liver cells is increased; it occurs in hepatocellular diseases because the critical load level of the liver cells (their ability to work) is diminished. In either case, because there is a differential in total load within the liver lobule, failure of bile secretion first occurs in the centrolobular zone. This is manifested histologically by centrolobular bile stasis. Our hypothesis indicates that cholestatic ("cholangiolitic") hepatitis need not necessarily result from intrahepatic biliary obstruction, as is commonly assumed, but may well be the result of failure of the liver cell itself.

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(Interlingua on page 80)

SUMMARIO IN INTERLINGUA

(See page 53 for original article)

Studios Relative Al Capacitate Diffusori Del Pulmon 1. Un Technica Simplificate Pro Le Retention Del Halito A Monoxydo De Carbon

Es describite un modification del test a retention de halito con monoxydo de carbon pro mesurar le capacitate diffusori del pulmon. Nitrogeno es usate in loco de helium como gas indicatori. Es discutite le importantia del relation inverse inter diffusion de CO e tension alveolar de O_2. A un tension de O_2 de 300 mm de Hg, le normal valor medie del diffusion de monoxydo de carbon in le pulmones es 16 ml per minuta per mm de Hg.

PULMONARY DIFFUSION CAPACITY STUDIES
I. A SIMPLIFIED TECHNIQUE OF CARBON MONOXIDE BREATHHOLDING

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The recent development of more precise methods of blood gas analysis (Riley, Proemmel and Franke[15]) and the availability of rapid gas analyzers have made possible the clinical determination of pulmonary diffusion capacity. The descriptive term "alveolar capillary block" (Austrian *et al.*[1]) is widely used in characterizing the altered pulmonary physiology of certain pathologic conditions of the lung. The diffusion capacity is now regarded as one of the two "really useful" tests[12] among the many available for appraising lung function.

Forster *et al.*[6], Marks *et al.*[13], and Bates and Pearce[2] have recently compared several diffusion capacity tests. The carbon monoxide breathholding technique is the simplest to perform. This paper will describe a modification of the breathholding technique which may be carried out by chemical analysis of air samples when electronic equipment is not available. Gas analyzers and a multichannel recorder add to the simplicity and accuracy of the test and, therefore, the description will be confined to the latter procedure.

Methods. The carbon monoxide breathholding test for diffusion capacity is based on the diffusion of a measured amount of CO from the alveolar air into the pulmonary capillary red cell hemoglobin during a period of approximately 10 seconds. This test, first described by August and Marie Krogh[10] in 1910, recently has been improved by Forster *et al.*[7,8]. The latter uses a 10% concentration of helium in the inspired gas to calculate the initial CO concentration of the alveolar sample. Since the residual volume must be determined in order to calculate the alveolar volume which is used in Krogh's equation, our modification is adopted to be carried out immediately following the completion of the open circuit functional residual capacity test. Nitrogen concentration is used instead of helium to determine the dilution of CO.

The open circuit method for determining functional residual capacity has been previously described (Curtis, Emanuel and Rasmussen[4], and Darling, Cournand and Richards[5]). At the completion of the 7-minute nitrogen washout test the patient is continued on 100% O_2, and requested to take several maxi-

mum inspirations and expire fully to further empty his less well ventilated spaces of residual N_2. The expiratory N_2 concentration is recorded from the nitrogen meter. Next the patient inhales from a volume recording spirometer a mixture of 0.4% CO in room air which he is instructed to hold in his lungs for 10 seconds, determined by a stop watch. The expired sample is then collected in another volume recording spirometer and the expired alveolar gas sample is withdrawn from the tubing near the mouthpiece into a Liston-Becker CO analyzer using a 10 inch absorption cell. This analyzer has negligible sensitivity to respiratory gases and is not sensitive to water vapor. A similar sample is withdrawn at the same time into the Waters' nitrogen meter. Records of time, volumes and concentration of CO and N_2 are made on a Sanborn polyviso recorder. The lag times in the analyzers are not a factor in determining the concentrations of the gases which are used to calculate the pulmonary diffusion capacity. A typical record of data obtained from a normal patient is illustrated in Fig. 1.

The patient may be turned directly back into the O_2 circuit and after several minutes the breathholding test repeated or different gas mixtures may be introduced for testing. Krogh[9] has shown that repeating several tests introduces no significant error from back pressure. Usually 2 or 3 tests are run and then the N_2 meter is standardized with 3 known gas concentrations to determine precisely the alveolar N_2 concentration. Neither of the analyzers are linear in the ranges used for this test. It is, therefore, necessary to make corrections. The nitrogen concentration is read as shown in Fig. 1, directly off the standardization line. The CO concentration is determined from a standard curve prepared with known concentrations of CO.

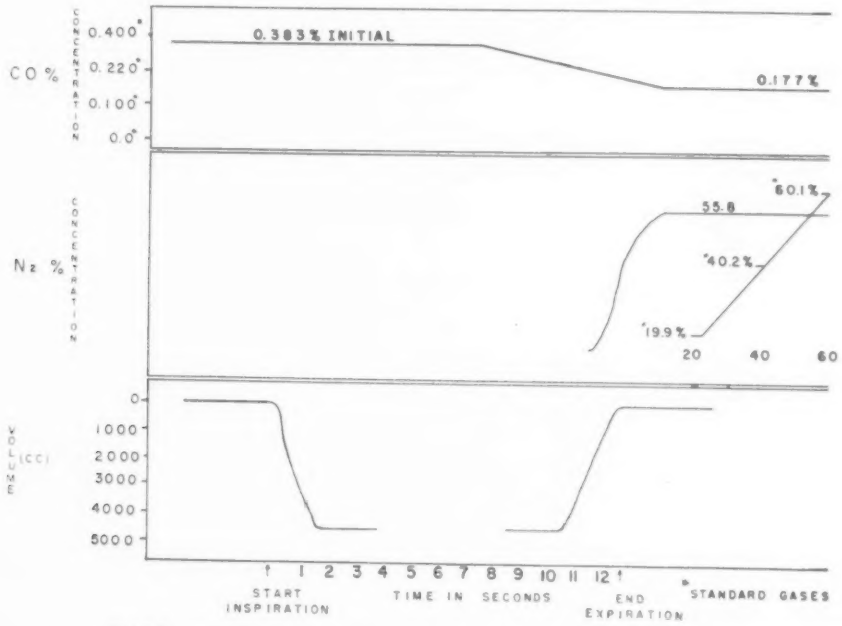

DIAGRAM OF TYPICAL RECORD OF DATA OBTAINED FROM A NORMAL PATIENT FOR CALCULATION OF CARBON MONOXIDE DIFFUSION CAPACITY

Fig. 1

Calculation of the pulmonary diffusion capacity is carried out in a manner similar to that described by Forster *et al.*[6] using the equation of Krogh. An example of our method of calculating D is provided in an addendum.

Results. Clinical results of this test are given in detail in the second article of this series. It will be noted that the normal diffusion capacity for carbon monoxide by this method is 16 ml. per min. per mm. Hg. This value is approximately half the normal value obtained by Ogilvie *et al.*[14] using helium as the dilution indicator gas. The difference is due to the oxygen tension in the alveoli at the moment the test is performed. With the patient breathing room air the alveolar tension of O_2 is approximately 100 mm. Hg. When the normal lungs are filled with oxygen one breath of inspired room air results in an alveolar O_2 tension of approximately 300 mm. Hg. Roughton[16], Forster, Fowler and Bates[7], Kruhøffler[11], Cander and Forster[3] and Ogilvie *et al.*[14] have shown the inverse relationship between CO diffusion and O_2 tension. At the alveolar O_2 tension level of 300 mm. Hg there is no significant difference between the results using helium or nitrogen as the indicator gas. The use of nitrogen has the following advantages: (1) Nitrogen is a physiologic gas and is available in room air at the concentration necessary for the test. (2) Nitrogen concentration is rapidly and accurately determined by an electronic nitrogen meter or by the Scholander microanalyzer. The chemical analysis for helium is laborious, the katharometer for measuring helium concentration is sensitive to the respiratory gases necessitating further analysis and corrections, the mass spectrometer is presently very expensive.

Summary. A modification of the carbon monoxide breathholding technique for measuring the diffusion capacity of the lung has been described. Nitrogen is used instead of helium as the indicator gas. The importance of the inverse relationship between CO diffusion and alveolar O_2 tension is discussed. At O_2 tension of 300 mm. Hg the average normal value for carbon monoxide diffusion in the lungs is 16 ml. per min. per mm. Hg.

ADDENDUM. The following example (Fig. 1) illustrates our method of calculating D_{co} in a manner similar to that described by Forster[6] using the equation of Krogh; all gas volumes indicated below have been reduced to standard temperature and pressure and corrected for water vapor:

$$(1)\quad D_{co} = \frac{\text{Alveolar volume} \times 60}{(\text{B-47 mm. Hg})\left(\begin{matrix}\text{Time in seconds}\\ \text{of Breathholding}\end{matrix}\right)} \times \text{Ln} \frac{\left(\begin{matrix}\text{Initial expired*}\\ \text{alveolar } CO\%_c\end{matrix}\right)}{\left(\begin{matrix}\text{Final expired}\\ \text{alveolar } CO\%_c\end{matrix}\right)}$$

(2) Alveolar volume = Inspired volume + Residual volume
Substituting with the appropriate data from Fig. 1 after reduction to standard conditions we have:

(2*a*) Alveolar volume = 3745 ml. + 1125 ml. = 4870 ml.

(3) Time of breathholding = 12.3 seconds

(4) (B-47 mm. Hg) = (744 mm. Hg—47 mm. Hg) = 697 mm. Hg

Initial expired alveolar $CO\%_c$ is obtained by calculating the dilution factor of N_2 by the residual air since the initial CO concentration is diluted to the same extent as the N_2. A correction of N_2 in the residual volume is determined before initiating the D_{co} test.

*The expression initial *expired* alveolar CO% follows Krogh's terminology. He actually determined initial expired alveolar CO% by obtaining a small sample of the patient's alveolar air before breathholding. Forster's method determines this factor by dilution of He and our method by dilution of N_2.

$$(5)\quad \left(\begin{matrix}\text{Corrected expired}\\ \text{alveolar } N_2{}^{C}_{c}\end{matrix}\right)=\left(\begin{matrix}\text{Observed expired}\\ \text{alveolar } N_2{}^{C}_{c}\end{matrix}\right)-\frac{\left(\begin{matrix}N_2{}^{C}_{c}\text{ in RV}\\ \text{before test}\end{matrix}\right)\text{X RV}}{\text{Alveolar volume}}$$

$$=55.8{}^{C}_{c}-\frac{(1{}^{C}_{c})\times(1125\text{ ml.})}{(4870\text{ ml.})}=55.6{}^{C}_{c}$$

$$(5a)\quad \left(\begin{matrix}\text{Initial expired}\\ \text{alveolar } CO{}^{C}_{c}\end{matrix}\right)=\frac{\left(\begin{matrix}\text{Corrected expired}\\ \text{alveolar } N_2{}^{C}_{c}\end{matrix}\right)}{\text{Inspired } N_2{}^{C}_{c}}\times(\text{Inspired } CO{}^{C}_{c})$$

$$(5b)\quad \text{Inspired } N_2{}^{C}_{c}=\text{Room air } N_2{}^{C}_{c}-\frac{\text{Room air } N_2{}^{C}_{c}\times\text{Inspired } CO{}^{C}_{c}}{100{}^{C}_{c}}$$

$$=79.0{}^{C}_{c}-\frac{79{}^{C}_{c}\times0.383{}^{C}_{c}}{100{}^{C}_{c}}=78.7{}^{C}_{c}$$

Substituting (*5b*) into (*5a*) we have:

$$\text{Initial expired alveolar } CO{}^{C}_{c}=\frac{55.6{}^{C}_{c}}{78.7{}^{C}_{c}}\times(0.383{}^{C}_{c})=0.271{}^{C}_{c}\text{ CO}$$

(6) Final expired alveolar $CO{}^{C}_{c}=0.177{}^{C}_{c}$
(This value is read directly from Fig. 1 and in this example has been corrected for nonlinearity of the CO analyzer from a standard curve prepared with known CO concentrations)

(7) Substituting (*2a*), (3), (4), (*5a*) and (6) in equation (1) we have:

$$D_{co}=\frac{4870\text{ ml.}\times60}{697\text{ mm. Hg}\times12.3\text{ seconds}}\times\text{Ln}\frac{(0.271{}^{C}_{c})}{(0.177{}^{C}_{c})}$$
$$=14.6\text{ ml./min./mm. Hg}$$

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(Interlingua on page 52)

PULMONARY DIFFUSION CAPACITY STUDIES
2. CLINICAL RESULTS USING A MODIFIED CARBON MONOXIDE BREATHHOLDING TECHNIQUE

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The preceding article of this series describes a simplified breathholding technique for determining the carbon monoxide diffusion capacity of the lungs. This modification uses nitrogen as the dilution indicator gas rather than helium. Advantages of this method are: (1) The procedure is accomplished in a steady state period directly after determining the functional residual capacity by the open circuit technique. (2) A graphic record of nitrogen concentration, carbon monoxide concentrations, volumes and time are made available on one record for analysis and comparison. (3) Nitrogen, a physiologic gas, is readily available at the desired concentration in room air and is easily analyzed by an electronic nitrogen meter or by the Scholander microanalyzer. This paper will describe the clinical results of diffusion capacity tests using this technique. Comparisons will be made also with the breathholding method of Forster *et al.*[8] using helium.

Material and Methods. Normal males between the ages of 26 years and 61 years (average 36 years) were selected as controls. Male patients in various stages of pulmonary tuberculosis were studied; many of these underwent pulmonary resection for removal of their major disease. In addition, patients referred for evaluation of pulmonary function with the following diagnoses were investigated: advanced emphysema, farmer's lung, silicosis and sarcoidosis. The vital statistics for these groups are shown in Table 1 and the number of patients in each group appears in the second and sixth columns of Table 2.

Each patient received chest roentgenograms and a battery of pulmonary function tests to evaluate the pulmonary status. The vital capacity and maximum breathing capacity were performed according to the methods of Baldwin, Cournand and Richards[1]. The timed vital capacity was determined by our method[7], for 1, 2 and 3 seconds, but only the 2 second is reported here. The single breath O_2 test was done by our modification of the method[5] described by Comroe and Fowler[4] and the values for the terminal 500 ml. (Curtis and Rasmussen[6]) also were reported. A number of patients received bronchospirometry with bilateral diffusion determinations, cardiac catheterization, oximeter studies and arterial blood gas analysis.

TABLE 1.—AVERAGE VALUES FOR PULMONARY FUNCTION TESTS ON 101 PATIENTS

	Age	*Height Inches*	*Weight Pounds*	*VC ml.*	*VC % of Normal*	*FVC 3 Sec. %*	*MBC L/min*	*MBC % of Normal*	*ERV ml.*	*RV ml.*	*TLC ml.*	*RV TLC × 100 %*	*Index Mixing %*	*O_2 Single Breath N_2 cc*	*O_2 Terminal N_2 %*
Normal or minimal tuberculosis	36	70	169	4718	112	91	156	120	1681	1705	6462	26	1.0	0.9	3.8
Moderately advanced tuberculosis	35	70	152	4092	97	81	128	100	1377	2051	6140	34	5.1	1.4	6.3
Far advanced tuberculosis	44	69	148	3046	77	73	76	70	1000	2067	5090	40	4.4	3.2	9.3
Emphysema	59	68	134	2665	73	51	46	47	950	4027	6695	60	8.5	7.9	9.3
Farmer's lung	43	69	152	3369	85	75	84	72	1056	2710	6130	44	4.4	4.0	6.9
Silicosis	53	67	144	2176	55	74	63	63	688	2524	4700	53	5.5	6.2	7.3
Sarcoid	35	70	165	4071	102	82	121	93	1459	1941	6229	33	3.0	1.9	5.0

These studies will not be reported in this paper. The basic pulmonary function data appears in Table 1. Table 2 contains the data on alveolar O_2 tensions and carbon monoxide diffusion capacity. Supporting data on several miscellaneous groups will be presented in the discussion.

Results. The results of the carbon monoxide diffusion capacity determinations are divided into 2 groups: (1) Medium O_2 tension range in which room air nitrogen was used to determine the dilution factor and (2) low O_2 tension in which the helium method was used. At medium O_2 tension (297 mm. Hg) the average carbon monoxide diffusion capacity for normal males is 16 ml. per min. per mm. Hg. At low O_2 tension (112 mm. Hg) the average diffusion capacity for the group of normals was 24 ml. per min. per mm. Hg. Figure 1 illustrates how the diffusion capacity for carbon monoxide varies with the alveolar O_2 tension. A few normal values for high O_2 tension (600 mm. Hg) are included to demonstrate the curve. The normal curve from Kruhøffer[12] has been added to the graph for comparison. He obtained this by an isotopic method which gives somewhat higher values probably due in part to an unsteady state produced by rapid deep breathing. The inverse relationship between the diffusion capacity for carbon monoxide and O_2 tension has been shown by Roughton[16] and others (Cander and Forster[2], Forster *et al.*[9] and Kruhøffer[12]) to be due to competition for the hemoglobin molecule by CO and O_2.

TABLE 2.—AVERAGE VALUE FOR CARBON MONOXIDE DIFFUSION ON 101 PATIENTS

	D_{co} *Medium* pO_2 (N_2)				D_{co} *Low* pO_2 (*He*)			
	No. of Patients	pO_2	D_{co} *ml./min./mm. Hg*	D_{co} *Range*	*No. of Patients*	pO_2	D_{co} *ml./min./mm. Hg*	D_{co} *Range*
Normal or minimal tuberculosis	14	297	15.9	11.0–19.8	10	112	23.9	14.5–30.1
Moderately advanced tuberculosis	9	309	17.2	5.8–26.5	2	120	22.3	21.1–23.5
Far advanced tuberculosis	34	336	11.5	1.2–22.4	15	104	14.6	4.0–28.1
Emphysema	21	425	7.3	0 –14.7	12	104	16.0	4.2–26.7
Farmer's lung	9	375	11.1	4.4–19.6	4	105	14.4	8.3–21.3
Silicosis	4	371	9.4	7.0–11.5	2	102	18.6	13.2–24.1
Sarcoid	10	323	15.3	6.6–21.8	7	106	24.0	13.4–29.8

Examination of Table 2 shows that patients selected in the groups with far advanced tuberculosis, advanced emphysema, farmer's lung and silicosis have reduced carbon monoxide diffusion capacities. Several of the severe sarcoid patients also had low values for diffusion, while those patients with little destruction of lung tissue were normal. Figure 2 illustrates results obtained in patients tested with both the helium and nitrogen methods at low and medium O_2 tension respectively. It

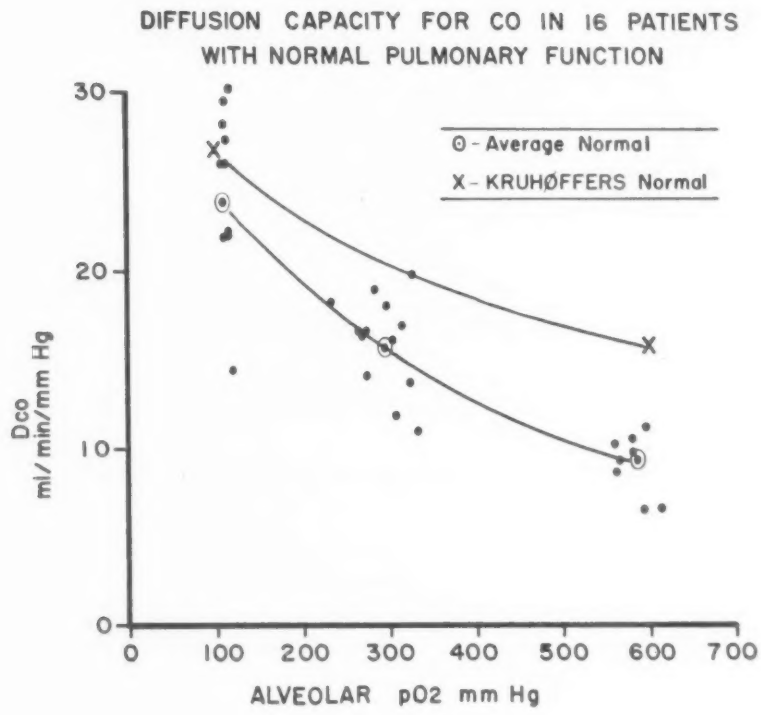
DIFFUSION CAPACITY FOR CO IN 16 PATIENTS
WITH NORMAL PULMONARY FUNCTION
O - Average Normal
X - KRUHØFFERS Normal
Dco
ml/ min/mm Hg
30
20
10
0
0
100
200
300
400
500
600
700
ALVEOLAR pO2 mm Hg

Fig. 1

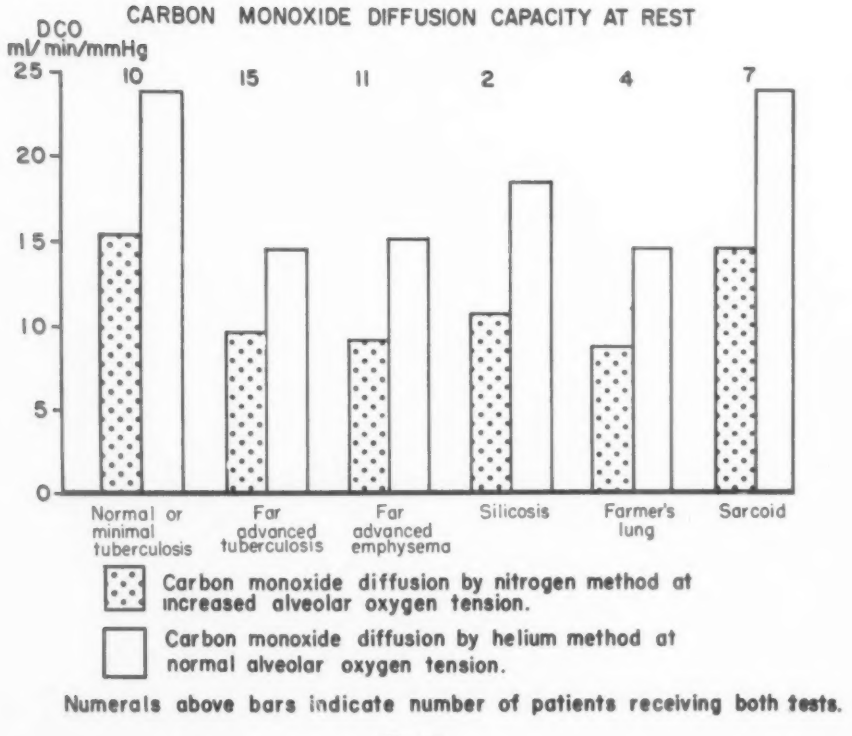
CARBON MONOXIDE DIFFUSION CAPACITY AT REST
DCO
ml/ min/mmHg
25
20
15
10
5
0
10
15
11
2
4
7
Normal or minimal tuberculosis
Far advanced tuberculosis
Far advanced emphysema
Silicosis
Farmer's lung
Sarcoid
Carbon monoxide diffusion by nitrogen method at increased alveolar oxygen tension.
Carbon monoxide diffusion by helium method at normal alveolar oxygen tension.
Numerals above bars indicate number of patients receiving both tests.

Fig. 2.

is seen that the nitrogen technique yields results consistent with the helium method, but lower, because of the differences in O_2 tensions in the 2 procedures.

Discussion. Forty-four years ago Marie Krogh[11] wrote, "What concerns us is not the exact figure for diffusion through a certain membrane, but the conditions determining the supply of oxygen to the blood." Recently efforts have been made to compartment diffusion by calculating the diffusion capacity of the alveolar capillary membrane (Dm) and the pulmonary capillary blood volume (Vc) (Forster *et al.*[10], Rankin, McNeill and Forster[15] and Roughton and Forster[17]). It must be emphasized that the parameters of pulmonary diffusion are numerous and heterogeneous, especially in diseased lungs complicated with emphysema. Review of the patients with abnormal function included in Table 1 showed that many have significant emphysema. Figure 3 shows that the higher the ratio of RV/TLC the more elevated the O_2 tension becomes under the conditions of this method. Our experience using nitrogen as the indicator gas suggests that at the higher O_2 tensions (in range of 600 mm. Hg) considerable error may be introduced by uneven distribution of gases in patients with advanced emphysema.

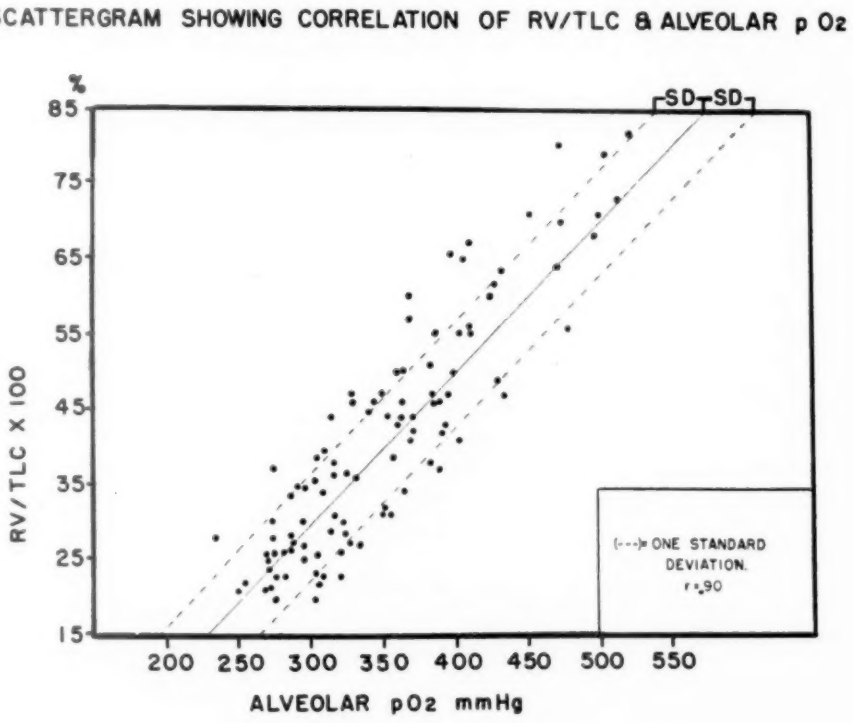

Fig. 3.

Krogh[11] also recognized that an increase in hemoglobin concentration or oxygen carrying capacity of the blood will improve the value for diffusion. At high O_2 tensions the blood compo-

nents of the pulmonary capillaries tend to be the rate limiting factor for the diffusion of carbon monoxide. At O_2 tensions of 300 mm. Hg the diffusion test is sensitive to hemoglobin concentration as illustrated in the following experiments. Two patients with low hemoglobin values were subjected to diffusion capacity tests before and after transfusions. Table 3 shows a definite increase in diffusion resulting from improvement of the anemia. Conversely, a patient with mild polycythemia and hemochromatosis was tested before and after phlebotomy.

TABLE 3.—THE EFFECT OF HEMOGLOBIN CONCENTRATION ON DIFFUSION CAPACITY

	Before Transfusion			*After Transfusion*			
Patient No.	*Hgb.*	*Alv pO_2*	*D_{CO}*	*Hgb.*	*Alv pO_2*	*D_{CO}*	*Pints Whole Blood*
72	8.6	385	4.3	13.0	388	9.7	+1
74	7.0	311	2.3	12.3	322	4.7	+1
				14.2	306	9.5	+1
	Before Phlebotomy			*After Phlebotomy*			
	Hgb.	*Alv pO_2*	*D_{CO}*	*Hgb.*	*Alv pO_2*	*D_{CO}*	
76	17.8	335	12.0	13.8	344	10.3	−2

Reduction in hemoglobin was followed by a fall in diffusion capacity (Table 3). Thus in evaluating diffusion capacity both alveolar O_2 tension and hemoglobin concentration should be known.

At low O_2 tensions the rate limiting factor for diffusion of carbon monoxide lies in the membrane component. Tests in very low O_2 tension ranges have not been possible. Comroe *et al.*[3], Ogilvie *et al.*[14] and McIlroy and Bates[13] have reported reduction in diffusion capacity following pneumonectomy. It is assumed that the loss is due primarily to the decrease in the area of the membrane through which diffusion takes place. However, on theoretic grounds more blood will be passing through the remaining lung which might improve the diffusion on this side. One would not expect the diffusion capacity to approach the preoperative level unless the removed lung was completely destroyed by disease. To investigate these considerations 6 patients were studied who underwent pneumonectomy for far advanced pulmonary tuberculosis. Table 4 shows the vital capacity, maximum breathing capacity, total lung capacity and diffusion studies before and after pneumonectomy. Also included are preoperative bronchospirometry studies on 3 of the patients. Patient 1 had a badly destroyed lung with almost no O_2 uptake indicating little blood flow through this side. Pneumonectomy did not change the diffusion significantly. On the other hand, the other patients had lesser amounts of disease in the removed lungs and hence diffusion capacity for carbon monoxide was significantly reduced. Inspection of Table 4 shows no striking correlation between reduction in ventilatory function and diffusion capacity following pneumonectomy. However, there is apparently a trend between O_2 uptake of the removed lung and the loss of diffusion capacity.

More definitive studies now are underway using bronchospirometry to obtain diffusion capacities for each lung separately.

Thus on the basis of the diffusion studies included in this report it would appear that the modified breathholding technique provides a reliable means of clinically evaluating the state of the pulmonary membrane-blood barrier to diffusion.

TABLE 4. PULMONARY FUNCTION DATA AND DIFFUSION CAPACITY FOR CARBON MONOXIDE ON 6 PATIENTS WITH FAR ADVANCED PULMONARY TUBERCULOSIS BEFORE AND AFTER PNEUMONECTOMY

							Bronchospirometry					
							Right			*Left*		
No.		*VC* %	*MBC* %	*TLC* *ml.*	*Alv.* pO_2 *mm. Hg*	D_{CO} *ml./min./mm. Hg*	*Vent* %	O_2 *uptake* %	*VC* %	*Vent* %	O_2 *uptake* %	*VC* %
(1)	before	64	62	4565	313	9.1	82	98	71	18	2	29
	after	41	51	3183	348	9.6						
(2)	before	91	99	4963	316	15.8	65	76	81	35	24	19
	after	52	84	2773	355	9.5						
(3)	before	69	81	4202	295	11.8	33	38	41	67	62	59
	after	36	50	3185	326	10.2						
(4)	before	63	70	3831	287	11.4						
	after	58	58	3334	280	9.7						
(5)	before	88	91	6400	328	13.4						
	after	67	65	3346	347	8.						
(6)	before	54	64	3400	325	17.3						
	after	43	49	2682	338	12.2						

Summary and Conclusions. A modification of the breathholding technique for carbon monoxide diffusion capacity of the lung is presented. The test is performed immediately after the open circuit determination of the functional residual capacity. Nitrogen is used in place of helium for calculating the dilution of carbon monoxide by the residual volume. At oxygen tensions in the average range of 300 mm. Hg the mean normal diffusion capacity for carbon monoxide is 16 ml. per min. per mm. Hg with a range of 11.0 to 19.8 ml. per min. per mm. Hg. The role of oxygen tension in modifying diffusion capacity is stressed. Likewise the amount of circulating hemoglobin is an important factor. Patients with far advanced tuberculosis, advanced pulmonary emphysema, and advanced stages of farmer's lung, silicosis and sarcoidosis may have low diffusion capacities. Data on patients are included to demonstrate changes in diffusion capacity following medical and surgical treatment.

ACKNOWLEDGMENTS: We are indebted to Dr. Helen A. Dickie, Dr. Edgar S. Gordon and Dr. Karver L. Puestow of the University Hospitals Staff for allowing us to test several of their patients with farmer's lung and emphysema. Dr. Robert E. Forster, Dr. John Rankin and Dr. A. B. Falcone critically reviewed our paper and gave us many useful suggestions. We wish to thank Mr. Forrest Fisher for preparing the illustrations, Miss Mary Kieffer and Mrs. Lila Mulloy for preparing the manuscript.

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SUMMARIO IN INTERLINGUA

Studios Relative Al Capacitate Diffusori Del Pulmon
2. Resultatos Clinic Obtenite Per Medio De Un Modificate Technica De Retention Del Halito A Monoxydo De Carbon

Es presentate un modification del technica de retention del halito pro determinar le capacitate diffusori a monoxydo de carbon in le pulmones. Le test es effectuate immediatemente post le determination a circuito aperte del capacitate residue functional. Nitrogeno es usate in loco de helium pro calcular le dilution de monoxydo de carbon per le volumine residue. A tensiones de oxygeno del ordine de 300 mm de Hg, le normal valor medie del capacitate diffusori pro monoxydo de carbon es 16 ml per minuta per mm de Hg, con valores extreme de 11,0 e 19,8 ml per minuta per mm de Hg. Es sublineate le rolo del tension de oxygeno in modificar le capacitate diffusori. Le quantitate del hemoglobina in circulation es etiam un factor importante. Patientes con avantiatissime tuberculose, avantiate emphysema pulmonar, e avantiate stadios de pulmon de fermero, silicosis, e sarcoidosis pote haber basse capacitates diffusori. Es includite datos que demonstra le alterationes del capacitate diffusori que occurre in patientes post lor subjection a therapias medical e chirurgic.

THE EFFECT OF FEVER AND CORTICOTROPIN ON THE *IN VIVO* DEGRADATION OF ALBUMIN IN MAN AS MEASURED WITH IODINATED HUMAN SERUM ALBUMIN

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and

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Patients with febrile illnesses frequently suffer from weight loss out of proportion to that expected from anorexia alone. The discovery that urinary nitrogen excretion increased during febrile illness was followed by the concept that such increased urinary nitrogen resulted from a toxic destruction of protein (Barr and DuBois[1], Cuthbertson and Tompsett[18], Grossman *et al.*[26], Howard[28], Moore and Ball[32], and Shaffer and Coleman[41]). Not only febrile illnesses associated with acute infections but burns (Croft and Peters[16]), radiation (Hall and Whipple[27] and Painter *et al.*[36]), nitrogen mustard (Blodgett *et al.*[7]), surgery (Moore and Ball[32]) and other traumata (Howard *et al.*[29]) also resulted in an increased urinary nitrogen excretion.

In the clinical ACTH conferences of 1951 and 1952 there were many reports delineating the effect of the adrenal cortex on the nitrogen loss accompanying stress reactions. In the first of these conferences a very rough correlation was found between nitrogen loss after trauma and heightened adrenal cortex activity (Browne *et al.*[12]). In the second conference were presented data showing that the administration of ACTH or cortisone was followed by increased urinary nitrogen (Bergenstal *et al.*[2]), at a time interval following the stimulus different from that which occurred when the increased urinary nitrogen followed trauma (Engel[21]).

Efforts to correct the toxic loss of protein through the use of high calorie dietary supplements were unsuccessful (Peters[37] and VanItallie *et al.*[47]) unless dietary supplementation was much greater than the calculated caloric deficit (Brunschwig, Clark and Corbin[14] and Mulholland *et al.*[33]).

The early studies in patients with infection (Barr and DuBois[1] and Shaffer and Coleman[41] indicated that during febrile relapse the nitrogen loss was not so great as during the initial attack, and that dietary supplementation during a relapse (that is, in a chronically ill patient) was partially to totally effective in maintaining nitrogen balance. Other workers (Browne, Schenker and Stevenson[13] and Moore and Ball[32]) emphasized the lessened nitrogen loss after trauma in the poorly nourished as compared to the well nourished individual, giving clinical corroboration to studies in the rat (Munro and Cuthbertson[34]). Furthermore, the magnitude of the nitrogen excretion was crudely related to the size of the stress; thus patients

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with traumatic fracture showed greater losses than those with a surgical osteotomy, following appendectomy there was a greater loss than following herniorrhaphy (Howard[28]). In general, less extensive surgery was accompanied by smaller nitrogen losses than more extensive surgery (Moore and Ball[32]).

urinary nitrogen rise (Bergenstal *et al.*[2]). Conversely, certain individuals who failed to show the expected urinary glucocorticoid rise after trauma did not have a rise in urinary nitrogen, although this was not an invariable finding (Browne *et al.*[12]). Finally, the degradation rate of iodinated albumin was shown to be unaffected by 40 mg.

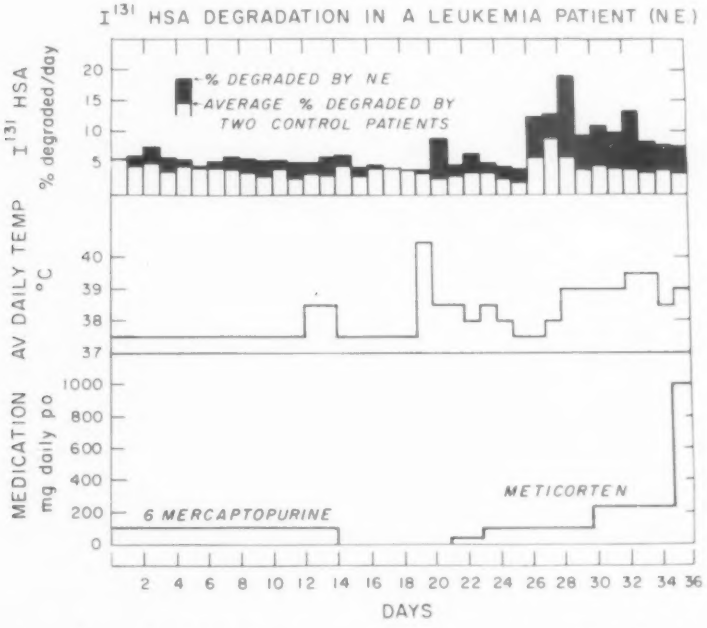

Fig. 1.—Thirty-six-day study of I^{131} albumin turnover in a patient in a "blastic" crisis of chronic myelocytic leukemia. In the lower part of the figure is shown daily intake of antileukemic agents. In the middle part of the figure is the average daily temperature and in the shaded upper part of the figure is the daily I^{131} albumin degradation rate for the study case, N.E. The upper limit of the unshaded area is the average degradation rate for the same I^{131} albumin preparation administered at the same time to a patient with chronic lymphocytic leukemia and to a control subject.

In similar fashion the nitrogen loss following ACTH administration was correlated with the ACTH dosage: 40 mg. daily for 5 days showed essentially no effect on nitrogen excretion (Forsham *et al.*[22]), 50 mg. daily for 5 days was accompanied by a 4-gm. urinary nitrogen rise and 70 mg. daily for 10 days resulted in an 8- to 10-gm.

daily of prednisone whereas 1000 mg. of prednisone doubled the degradation rate (Steinfeld and Watkin[44]).

Since serum albumin has been reported to decline following fever, trauma and other stress states (Cuthbertson[17], Gjessing and Chanutin[25], Luetscher[31], Peters[37,38], and Taylor, Mickelsen and Keys[46]) in contrast to

elevations in the serum alpha globulin and fibrinogen, it seemed a not unreasonable inference to place albumin among the proteins that participate in the "toxic destruction" following stress. Emphasis, however, must be directed to the conflicting reports in the literature on the decline of serum albumin after stress. Moore and Ball's[32] protocols do not support such a fall in serum albumin, Peter's series[37] is selective and Cuthbertson and Tompsett's series had only 6 cases[18]. Our own experience is not consistent on this point either, since some patients had an immediate fall in albumin concentration while others did not. While in general, serum albumin concentration reflects total body albumin, there is not a perfect correlation, so that changes in plasma volume or hydration might well account for differences observed.

The present attention to the behavior of iodinated human serum albumin (IHSA) during stress is the result of experience with patient N.E., a 36-year-old woman in a "blastic crisis" of chronic myelocytic leukemia (Fig. 1). During a febrile period accompanying a *Pseudomonas aeruginosa* septicemia, the patient's degradation rate of IHSA was twice that obtained at the same time with the same iodinated albumin preparation in 2 control patients. The addition of Meticorten in increasing doses added further to the accelerated catabolism of the iodinated albumin.

In an attempt to define the separate roles of fever and corticosteroids on the degradation rate of albumin the present study was undertaken.

Methods. The iodinated human serum albumin (IHSA) used in these experiments was prepared by a method which has been shown to achieve labeled protein of relatively uniform metabolic activity with a biologic half-time in the normal adult of about 17 days (Steinfeld *et al.*[43]). Patients at the National Cancer Institute, who were in a stable clinical status were selected (Table 1). Several patients had lost weight prior to the period of study. IHSA was delivered intravenously via calibrated syringes. Urine was collected in 24-hour pools into jars containing a basic iodide carrier solution. Blood specimens were obtained daily or 3 times weekly throughout the study. All radioactivity assays were performed using a Thallium activated sodium iodide scintillation well counter. Calculations were performed as follows:

$$\text{Per cent degraded per day} = \frac{\text{Fraction of the injected radioactivity in a 24-hour urine specimen}}{\text{Average fraction of the injected dose remaining in the body during that 24-hour period;}}$$

where the fraction of the injected radioactivity in a 24-hour urine specimen $=$

$$\frac{\text{Total counts injected, urine specimen}}{\text{Total counts injected}}$$

and the average fraction of the administered dose remaining in the body during that 24 hours = 1.00 minus the average of the cumulative daily fractions of the injected dose excreted at the beginning and end of the day in question.

All radioactive specimens were counted to within 1½% standard deviation in duplicate against appropriate standards. Known losses of urine occurred 3 times during the entire study and corrections based on daily creatinine excretions were applied. Patient J.O. vomited on 5 days; the vomitus was saved and the radioactivity of the vomitus was added to the urine radioactivity for that day.

A systematic error in the cumulative per cent excretion would seriously affect the per cent metabolized per day as calculated above. This was searched for by examination of the shape of the curve defined by the ratio of the fraction of the administered dose in 1 ml. of plasma divided by the fraction of administered dose remaining in the body. Ideally, this should come to a constant value if a systematic error did not occur (Berson *et al.*[3,4]). In Fig. 2, a representative graph of a control patient J.D. illustrates that no such regular error entered our data. In Fig. 2, Curve B is a plot of the fraction of the administered dose in 1 ml. of plasma as a function of time. IHSA is presumed to have achieved a steady state in its distribution throughout the body when Curve B becomes linear. This implies that degradation of the IHSA proceeds by the equation $A_t = A_0 e^{-kt}$

TABLE 1.—PERTINENT DATA ON INDIVIDUALS STUDIED

Patient	*Primary Diagnosis*	*Secondary Diagnosis*	*Weight in kg.*		*Clinical Status*	*Body Habitus*	*Proteinuria*	*Medication*
			time of study	*loss since disease onset*				
N. E.	Chronic Myelocytic Leukemia	Pseudomonas Septicemia	45	0	Terminal	Average	0	6-mercaptopurine Prednisone
D. L.	Bladder Cancer	Chronic Pyelonephritis	45	10	Stable	Thin		0
H. M.	Esophageal Cancer	———	50	5	Stable	Thin	0	Radiotherapy (Long term study)
A. H.	Diabetes Mellitus	Low grade Osteomyelitis	76	5	Stable	Moderately Obese	0	Insulin, Tetracycline
J. R.	Bladder Cancer	———	65	7	Stable	Average	0	0
J. O.	Nasopharyngeal Cancer	———	70	6	Stable	Muscular	0	Radiotherapy (Long term study)
C. J.	Hodgkin's Disease	Salmonella Carrier	52	16	Stable	Thin	0	Tetracycline
C. B.	Mycosis Fungoides	———	73	12	Stable	Moderately Obese	0	2% Hydrocortisone Skin Ointment

where A_t = amount of IHSA in 1 ml. of plasma at any time, t; A_0 = the initial amount in 1 ml. of plasma (as extrapolated from the straight line portion of the curve to "zero time;" k = the degradation constant; t = time. The initially more rapid decline of Curve B before it becomes linear is taken to reflect the sum of the degradation and exchange with other body compartments. Curve A shows the per cent of IHSA remaining in the body as a function of time. This is obtained by subtracting the cumulative daily per cent of the radioactivity excreted in urine from 100%. Curve C is the ratio of B over A. Should urine collections be incomplete B would decline faster than A, and C would slowly decrease. A constant value for C implies that loss from the plasma and loss from the body proceed at the same rate, as would be expected in a steady state. This is confirmation of the completeness of the urine collection.

In 5 patients fever was induced by the intravenous injection of typhoid H antigen, 100,000,000 organisms per ml. Doses varied between 25,000,000 and 200,000,000 organisms per day. Fever was not induced until such time as distribution of the IHSA was completed through the body compartments (Berson *et al.*[3,4]); this was determined by inspection of the plasma and distribution curves, assuming distribution to be completed when the straight line function on semilog paper appeared.

Fever in patient C.J. (Hodgkin's disease) was not induced, but appeared spontaneosly. Physical examination, roentgenograms and bacteriologic studies of the blood, urine and throat failed to reveal a specific febrile pathogen; an asymptomatic carrier state with *Salmonella tennessee* (C_t) was present throughout the study.

Daily fever units are defined as the number of degrees above 38° C. multiplied by 2 and by the number of hours elevated (temperatures taken hourly rectally). In patient J.O. the base line temperature was close to 36° C., and his fever units were calculated with

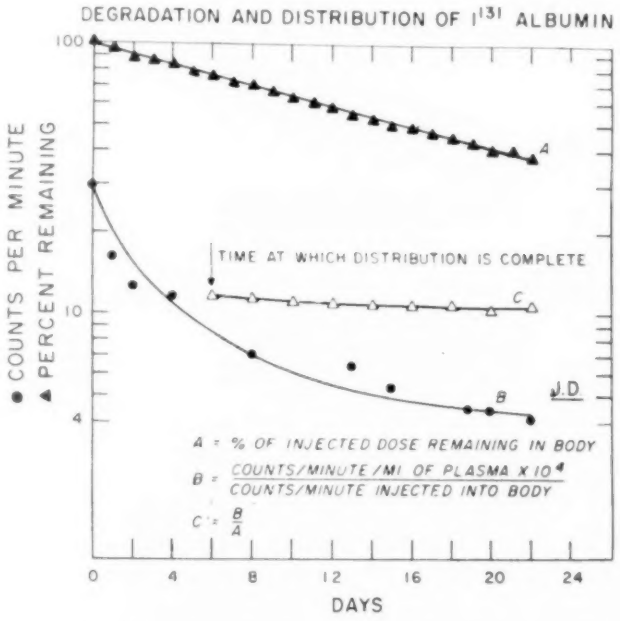

Fig. 2.—Graphical presentation of results of a typical study. *A* is a semilog plot of the per cent of the injected I^{131} albumin remaining in the body at any time. *B* is a semilog plot of the time course of the fraction of the injected I^{131} albumin in a ml. of plasma. *C* is the ratio of *B* to *A*, called the distribution curve (see discussion in text and reference 4).

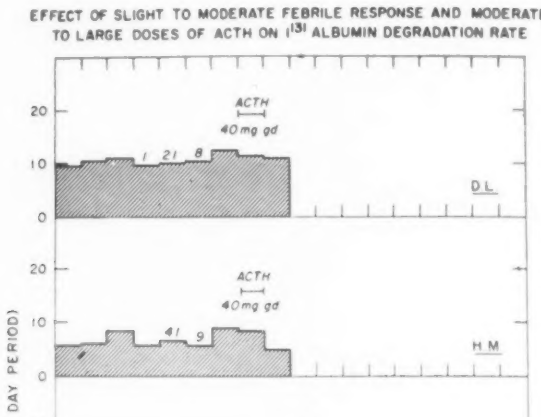

Fig. 3.—Degradation rate for I^{131} albumin in 2-day periods is shown by shaded areas with the per cent of the IHSA degradation on the ordinate. Number above shaded areas refer to total fever units (see text) for the 2-day period. Days and dosage of ACTH administration are indicated above the degradation rates for those periods.

the base line being 37.5° C. rather than 38° C. These units are reproducible to ± 20% by different observers.

ACTH. Intravenous or intramuscular ACTH was administered in varying doses daily for from 2 to 5 days in 5 different patients. ACTH administration followed the administration of typhoid vaccine in all instances by at least 4 days.

Results. For clarity of presentation data on IHSA degradation are given in terms of 2- and 4-day periods although urinary collections were terminated each 24-hour period. In Figs. 3 and 4 are shown the per cent of IHSA degraded in 2-day periods for patients D.L., H.M., J.R., A.H., J.O., and C.B., and in 4-day periods for patient C.J. Changes of more than 10% of the control degradation rate in the 2-day periods and 8% in the 4-day periods are considered significant. In Fig. 3 are the data on 5 patients who developed

fever following typhoid H antigen and who subsequently received ACTH. The amount of fever produced and the amount of ACTH given varied among the patients both in the amounts per day and in the number of days such stimuli were received. ACTH affected the degradation rate of IHSA in patient

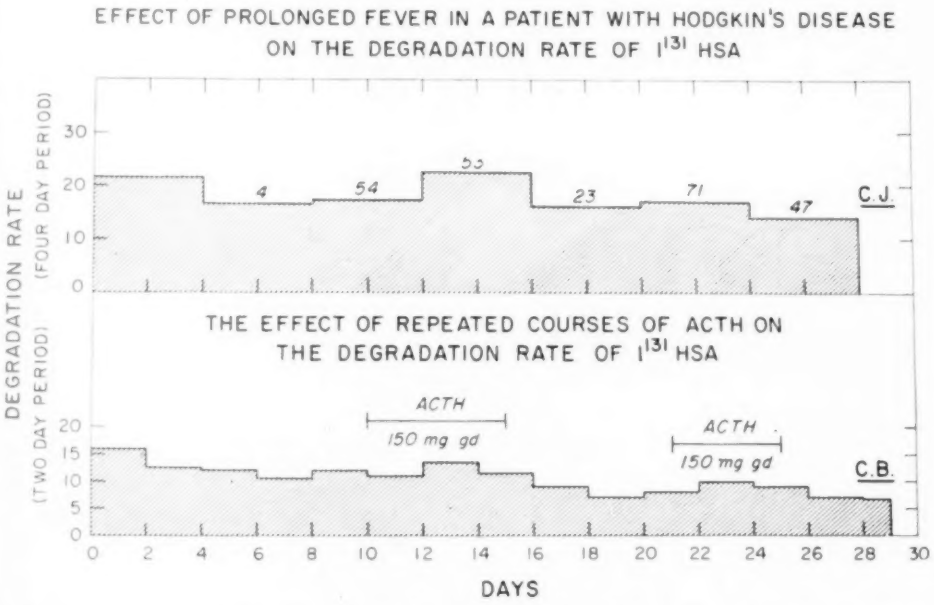

Fig. 4.—Upper part of figure: Degradation rate for I^{131} albumin in 4-day periods for a patient with spontaneous fever of Hodgkin's disease. Degradation rate in per cent is on ordinate. Numbers above shaded areas represent total fever units for the 4-day period. Lower part of figure: Degradation rate of I^{131} albumin in 2-day periods in a patient who received 2 courses of ACTH.

J.O. and A.H., with little effect apparent on IHSA degradation in the other patients.

In Fig. 4 are data on C.J., a patient with a Pel-Ebstein fever of Hodgkin's disease and C.B., a patient who received a 5-day and, later, a 4-day, course of ACTH. Both the fever in the patient with Hodgkin's disease and the course of ACTH were accompanied by rates of IHSA degradation greater than that seen in control individuals. In each patient samples from 24-hour urine collections were obtained for nitrogen and hydroxycorticoid determinations during control and experimental periods. All patients responded to both fever and ACTH with increased nitrogen excretion; the urinary corticoids were significantly increased above the control values following the ACTH, but not during the febrile periods.

The data on IHSA degradation from 5 of the 7 patients are re-plotted in Figs. 5 and 6 where only the degradation rates on days of and relevant to the experimental periods are charted. The numerals below the bar graph refer to the days after the injection of the IHSA on which the average is based. The numerals above the bar graph represent the average daily

number of fever units or milligrams of ACTH given for the indicated period. Control periods beginning several days after the injection of IHSA rather than including the first several days of the study were used for comparison with the experimental periods.

Although the preparation of IHSA used in this study was usually degraded at a constant rate after administration to man when the data are plotted as in Fig. 2, the various lots used had slight differences in this regard (Steinfeld *et al.*[43]). Not infrequently a daily variation in the metabolic rate with time was noted (Fig. 7); however expression of the data as in Fig. 2 results in smoothing out of small daily irregularities. It was, therefore, decided to compare a febrile or ACTH period with the control period just preceding and just following the particular stress rather than with an average degradation rate derived from control periods

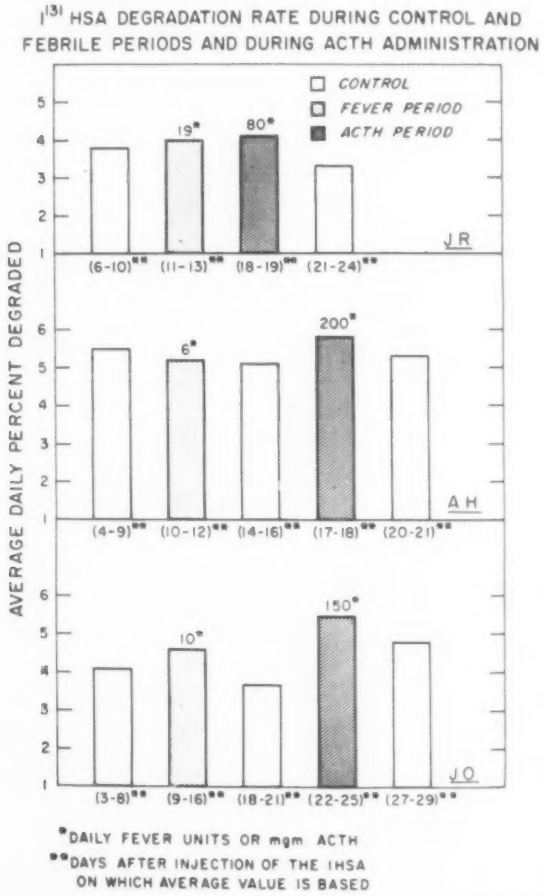

Fig. 5.—Bar graph presentation of changes in IHSA degradation subsequent to fever induction and ACTH administration.

throughout the period of the study. Since high doses of ACTH have effects lasting more than one day and a similar possibility exists for the metabolic effects of fever, the day following the febrile period of ACTH period was omitted in computing a control period average. Further, since high degradation rates are frequently noted in the first 24 to 96 hours after injection of IHSA (presumably related to partially denatured proteins during manufacture and their more rapid degradation *in vivo*), the first control period was selected beginning between the 3rd and 5th day, each particular decision being based on inspection of the data.

Only the first control period of J.R. was used because of a fever of 38° C. which occurred spontaneously between his induced febrile and his ACTH period. Only the first 3 febrile periods in C.J. were plotted because of a declining IHSA degradation rate after a prolonged stress.

Because of technical difficulties the

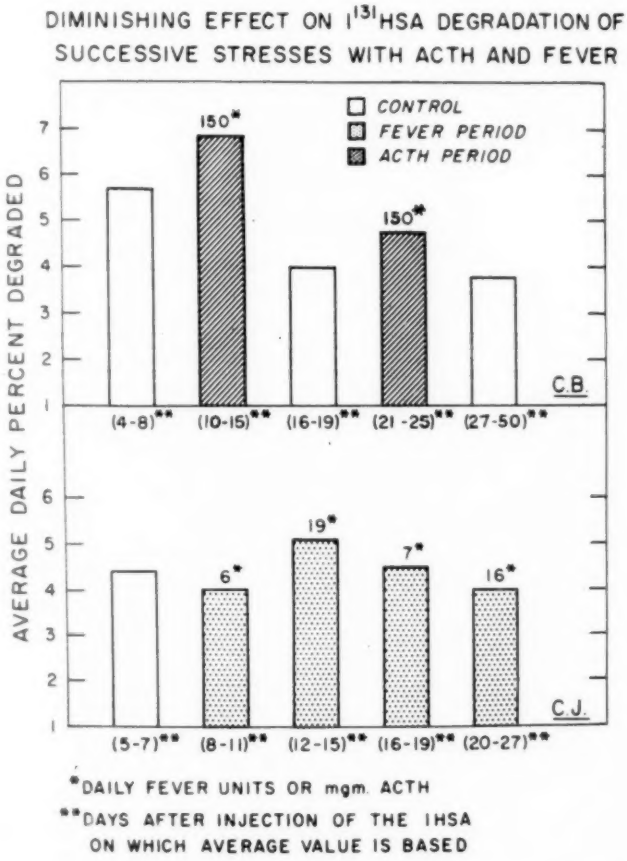

Fig. 6.—Bar graph presentation of effects of successive bouts of fever or successive courses of ACTH on degradation rate of I^{131} albumin.

febrile periods of D.L. and H.M. were compared only to a preceding control period rather than to a preceding and succeeding one, even though this may have obscured an effect of the fever on the degradation rate should the base line rate have been declining less elevation of the IHSA degradation rate than a first course; similarly prolonged fever is seen to be associated with diminishing effect on the rate of I^{131}albumin metabolism, although an early high and prolonged fever increases the degradation rate.

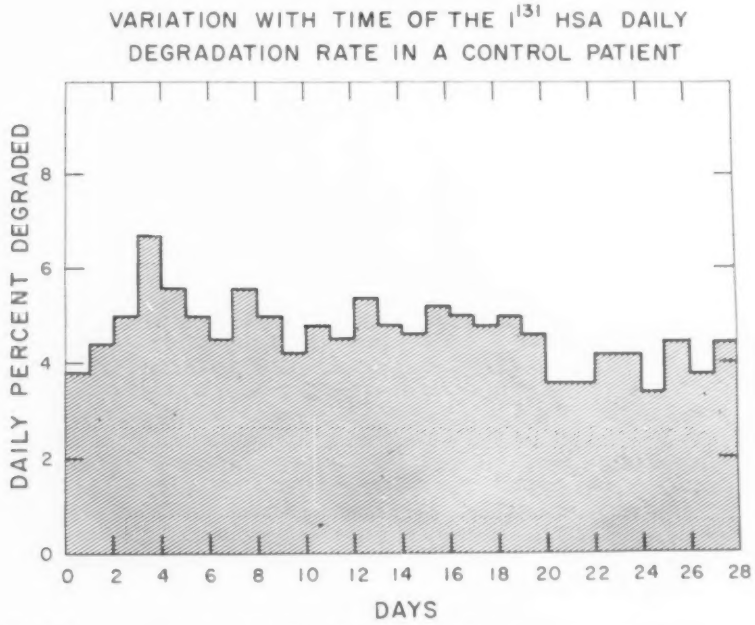
VARIATION WITH TIME OF THE I131 HSA DAILY DEGRADATION RATE IN A CONTROL PATIENT
DAILY PERCENT DEGRADED
0
2
4
6
8
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28
DAYS

Fig. 7.—Shaded area represents daily I^{131} albumin degradation rate based on urinary excretion of iodide, expressed as per cent of I^{131} albumin remaining in body. Subtracting cumulative per cent urinary I^{131} excretion from 100 and plotting on semilog paper (Fig. 3A) yields a single exponential although in fact there may be daily variations either in the body's metabolism of I^{131} albumin or in excretion rates of I^{131} and others.

during the febrile period (a circumstance which would be detected from examination of the subsequent control period).

In Fig. 5, ACTH is shown to produce regularly an elevation in the degradation rate of IHSA at the dosages given. Similarly, the prolonged fever of patient J.O. is associated with an obvious increased rate of iodinated albumin breakdown.

In Fig. 6, a second course of ACTH is demonstrated to be associated with

In Table 2, control degradation rates are presented for febrile and ACTH periods of each patient. These control rates are derived by averaging the degradation rates for control periods immediately before and immediately after either the febrile period or the ACTH period. The control rates so derived are then compared on a percentage basis to the degradation rate in the febrile and ACTH period, with successive febrile periods being selected in patients C.J. and J.O. and

successive ACTH periods in patient C.B. The total fever units in the febrile period (the average daily fever unit times number of days) and the total milligrams of ACTH given (the total daily dosage of ACTH times the number of days ACTH administered) are also indicated. Figure 8 is a plot on semilog paper of the per cent change in the degradation rate compared to the control periods against total ACTH dosage. An increase of the degradation rate of IHSA with increasing ACTH dosage is seen; there was no relationship between fever units and degradation rate on a similar plot.

The data presented refer only to I^{131} albumin degradation and not to breakdown of body albumin in terms of grams. Daily estimations of serum albumin concentration would permit conversion of the fractional turnover rates into grams of albumin catabolized or synthesized each day, or both, whether the patient was in a steady state or not. Unfortunately, changes in plasma volume subsequent to fever induction or ACTH administrations combined with an estimated 10% error in duplicate albumin determinations made data on stable albumin turnover uninterpretable. The changes in I^{131}albumin degradation found in this study, however, were significant as reflections of fractional turnover of total body albumin pools.

TABLE 2.—EFFECTS OF FEVER AND ACTH ON IHSA DEGRADATION RATE

ACTH

	Per Cent I^{131} HSA degraded			
			Total Units	
Patients	*Control*	*Experimental*	*ACTH*	*% Change*
J.O.	4.2	5.5	600	30.9
C.B.	4.0	5.0	650	25.0
	4.9	6.3	750	28.5
H.M.	3.5	4.4	80	25.7
A.H.	5.2	5.8	400	11.5
J.R.	3.7	4.1	160	10.8
D.L.	5.8	5.7	80	−1.7

FEVER

	Per Cent I^{131} HSA degraded			
Patients	*Control*	*Experimental*	*Fever Units*	*% Change*
J.O.	3.9	4.7	52	20.5
C.J.	4.4	5.1	76	15.1
J.O.	3.9	4.4	29	12.8
J.R.	4.0	4.0	58	0
A.H.	5.4	5.2	18	−3.8
D.L.	5.2	5.0	30	−3.9
H.M.	3.2	3.0	50	−6.9
C.J.		4.0	24	−9.1
	4.4	4.5	28	2.2

Discussion. In patient N.E. (Fig. 1) a rapid degradation rate of IHSA might have been due to the leukemic process, an associated septicemia with fever, or

prednisone therapy. The purpose of the present investigation was 1) to ascertain the effects of noninfectious fever on the metabolism of albumin and 2) to measure the effect of ACTH administration upon albumin metabolism.

Fever induced by typhoid H antigen was accompanied by increased rates of IHSA degradation (Fig. 5). Similarly ACTH administration was accompanied by increased IHSA degradation

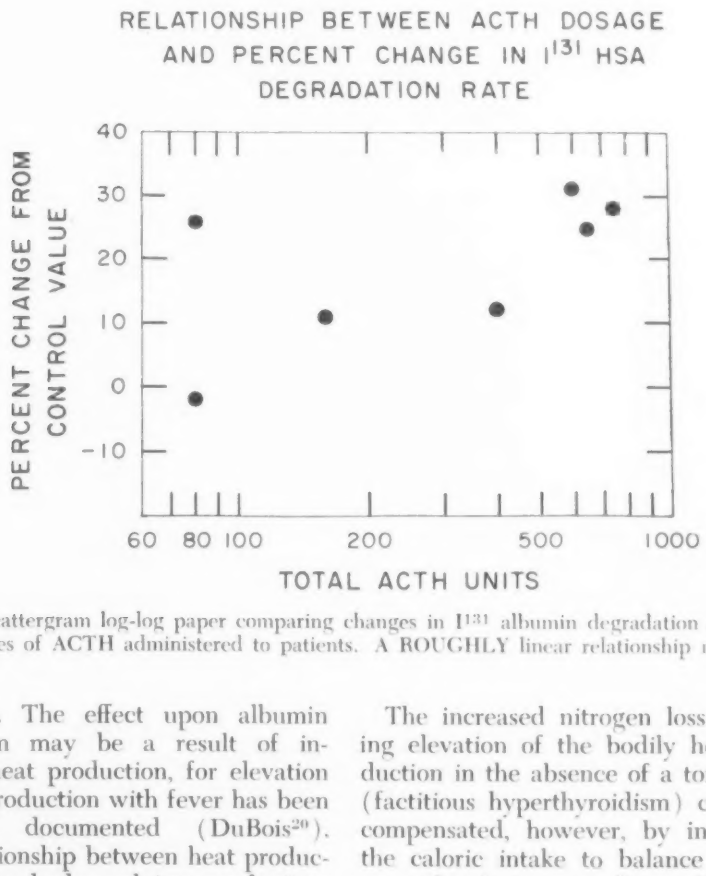

Fig. 8.—Scattergram log-log paper comparing changes in I^{131} albumin degradation rate with total dosages of ACTH administered to patients. A ROUGHLY linear relationship may exist

(Fig. 5). The effect upon albumin catabolism may be a result of increased heat production, for elevation of heat production with fever has been carefully documented (DuBois[20]). The relationship between heat production of the body and increased nitrogen excretion was studied using nitrogen balance following thyroxin administration (Boothly *et al.*[9]). These studies demonstrated increased nitrogen excretion associated with an accelerated metabolism. The relationship of albumin metabolism to heat production has been examined indirectly by studying the I^{131} albumin degradation rates in mammals in a wide range of sizes, and an inverse correlation was noted between body size and albumin turnover (Dixon, Maurer and Deichmiller[19]), and a direct correlation between albumin turnover and heat production (Spector[42]).

The increased nitrogen loss following elevation of the bodily heat production in the absence of a toxic state (factitious hyperthyroidism) could be compensated, however, by increasing the caloric intake to balance the increased caloric expenditure (Boothby *et al.*[9]), in contrast to the findings already cited that nitrogen loss after trauma is balanced with difficulty, if at all (VanIttalie *et al.*[47]). Such a "controllable" nitrogen wasting may repre-

sent a higher rate of the general level of nitrogen metabolism in accord with the Vant Hoff constant (DuBois[20]) rather than a toxic effect on protein *per se*.

As it appeared that nitrogen metabolism following trauma was different from that following an increase in heat production alone, the catabolic and anabolic phases of injury were advanced as an explanation (Browne, Schenker and Stevenson[13]). Increasing knowledge of the adrenocorticoid hormones' biologic actions and the experimental demonstration that the adrenal glands were not necessary for increased nitrogen excretion to occur following trauma (Ingle, Ward and Kuizenga[30] and Noble and Toby[35]) was in accord with the failure to correlate nitrogen loss following trauma to the appearance of heightened adrenal activity (Browne *et al.*[12]). Concepts of (A) anti-anabolic actions of the adrenocorticoids, based on demonstrations of growth inhibition (Blodgett *et al.*[7]), and (B) catabolic actions, based on urinary nitrogen increases with cortisone administration to starving animals (Cannon, Frazier and Hughes[15]) and compatible tissue protein kinetics (Fritz[23]) were simultaneously advanced. That both these aspects of anti-anabolism and catabolism may be related simply to an accelerated level of protein reactions generally was recently suggested by Geiger[24] and seems consistent with the lectures by Schoenheimer[10]. Nitrogen loss occurs not only following trauma and adrenocorticoid administration separately, but the physiologic response to trauma may be mediated in some measure via the adrenal glands.

Efforts to delineate further the kinetics of this process have been made recently. The first such studies in burned patients using labeled methionine (Blocker *et al.*[5]) and iodine labeled protein (Blocker *et al.*[6]) were not interpretable because of methodological errors. Surgical patients were studied with IHSA (Sterling, Lipsky and Freedman[45]) and the results suggested an increase in the postoperative rate of degradation of the albumin, although unfortunately the data were from blood curves exclusively rather than with simultaneous examination of the radioactivity appearing in the urine. A similar study on the effect of hydrocortisone on the IHSA metabolism using blood radioactivity curves alone was suggestive of an accelerated breakdown of the labeled protein (Blythe *et al.*[8]).

The experimental studies of Yuile *et al.*[48] in dogs with turpentine abscesses showed an acceleration in the rate of metabolism of the albumin, globulin metabolism being unchanged.

Fever may affect albumin degradation indirectly through changes either in hepatic blood flow or in the hepatic parenchyma, or both. Bradley and Conan[10,11] found increased hepatic blood flow during pyrogenic reactions. The increase in blood flow was accompanied by diminished Bromsulphalein extraction by the liver. When the febrile phase of the pyrogenic reaction to foreign proteins was inhibited by antipyretics, there was no corresponding effect on Bromsulphalein retention or liver blood flow. That is, in the absence of fever, liver blood flow was nonetheless increased and Bromsulphalein extraction decreased, indicating that the effect of foreign protein injection on the hepatic parenchyma and blood flow was not dependent on the fever produced. In the present study the increased IHSA degradation may have followed suppression of albumin synthesis by the liver (resulting from fever or ACTH), however the rate of degradation following the stresses applied here is greater than could be

accounted for by a feed-back mechanism, operating to keep daily albumin degradation constant, despite changes in the rate of synthesis.

In the present study an acceleration in the degradation rate of IHSA occurring with fever and during ACTH administration was found. The increased rate of degradation is roughly proportional to the size of the ACTH stress. The data on repeated fevers and on repeated ACTH stimuli (Fig. 6), although pointing to the possibility that repeated stresses of equal magnitude closely related in time to one another produced successively lessened effects in increasing the rates of metabolism of albumin are not considered conclusive.

Accelerated breakdown of IHSA following fever or ACTH stress does not imply catabolism is occurring at the expense of anabolism or to the exclusion of anti-anabolism. Since the iodine label is not re-utilized, hydrolysis of the peptides containing the iodine label would necessarily give rise to excretion of the tracer, whereas with native protein, hydrolysis of the peptides might be followed by re-utilization of the amino acids either in whole or in part (Schoenheimer[40]). Thus, although an increased rate of degradation occurs, inferences regarding the body balance of native proteins cannot be drawn. That is, a steady state may still exist although the exchange rate between compartments may be accelerated. The work of Yuile *et al.*[48], however, already cited, shows that in his experiments there is indeed a net loss to the body of albumin, rather than simply an acceleration of an already existing steady state. Since the label used was carbon-14, this label was available for re-utilization should re-utilization have occurred.

Summary. 1. The present concepts relating to the "toxic" destruction of protein are reviewed.

2. An increase in the "*in vivo*" degradation rate of iodinated human serum albumin occurred when the individuals under study had fever induced following typhoid H antigen.

3. A spontaneous fever occurring in a patient with Hodgkin's disease was associated with a greater iodinated albumin degradation rate than that noted when the patient was afebrile.

4. An increased rate of iodinated albumin degradation was noted following parenteral ACTH. This increase was roughly proportional to the amount of ACTH administered.

5. Suggestive evidence that a repeated fever or repeated courses of ACTH have less effect on increasing IHSA degradation than a first course is presented.

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SUMMARIO IN INTERLINGUA

Le Effecto De Febre E De Corticotropina Super Le Degradation *In Vivo* De Albumina Human, Mesurate Per Medio De Iodate Albumina De Sero Human

1. Es presentate un revista del conception currente de destruction toxic de proteina.

2. Occurreva un acceleration del degradation *in vivo* de iodate albumina seral human quando le individuos studiate habeva febre inducite per antigeno H a typhoide.

3. Febre spontanee, occurrente in un patiente con morbo de Hodgkin, esseva associate con plus intense grados de degradation de iodate albumina que lo que esseva notate quando le patiente esseva libere de febre.

4. Acceleration del degradation de iodate albumina esseva notate post administrationes parenteral de ACTH. Iste acceleration esseva grossiermente proportional al quantitate de ACTH administrate.

5. Es presentate observationes que pare significar que un repetite febre o repetite cursos de ACTH ha decrescente effectos augmentatori super le degradation de iodate albumina seral human.

SUMMARIO IN INTERLINGUA

(See page 45 for original article)

Un Explication Pro Le Localisation Centrolobular Del Stase Intrahepatic De Bile Que Occurre in Acute Morbos Hepatic

Iste articulo presenta un hypothese pro explicar le localisation centrolobular del intrahepatic stase biliari que occurre in le phase acute de morbos hepatic tanto obstructive como etiam hepatocellular. Le hypothese se basa super reflexiones relative al dynamica del fluxo biliari intra le lobulo hepatic. Nostre observationes indica (1) que le carga mechanic del fluido total in le cellulas hepatic tende a ager maximalmente al centro e minimalmente al peripheria del lobulo hepatic e (2) que le cellulas hepatic es capace a responder a un augmentate carga per un augmentate rendimento de energia pro le mantenentia del fluxo biliari usque un certe "nivello critic" es attingite. Disfallimento del secretion biliari resulta quando le carga total que age super le cellulas hepatic excede le nivello critic de ille carga. Iste disfallimento occurre in jalnessa obstructive proque le carga total super le cellulas hepatic es augmentate; illo occurre in morbos hepatocellular proque le nivello critic del carga in le cellulas hepatic (*i.e.* lor capacitate de labor) es reducite. In ambe casos, le disfallimento del secretion biliari occurre primo in le zona centrolobular in consequentia del facto que le carga total non es identic in omne partes del lobulo hepatic. Isto se manifesta histologicamente per le stase centrolobular del bile. Nostre hypothese indica que hepatitis cholestatic ("cholangiolitic") non resulta necessarimente de un obstruction intrahepatic del bile (como on lo suppone communmente) sed pote ben resultar de dysfunction del cellula hepatic mesme.

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MEDICINE

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DIAGNOSTIC METHODS IN INTESTINAL MALABSORPTION

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THE malabsorption syndrome has recently been the subject of wide interest[1,121,155]. Difficulties in exact diagnosis probably account for the infrequent recognition of the disease. Recent advances have led to effective therapy[32,121], and this has emphasized the importance of diagnosis. At the same time many new absorption tests have appeared. The present review consists of an evaluation of the methods employed in the diagnosis of intestinal malabsorption.

Diminished absorption from the intestinal tract may result from insufficient pancreatic lipase or bile salts, pathologic involvement of the small intestine, and surgical procedures which remove important portions of the gastrointestinal tract or create certain anastomoses. A large group of patients remain, however, in whom the etiology of intestinal malabsorption is unclear. The fact that our knowledge of the normal processes of intestinal absorption is superficial accounts for our lack of understanding of the pathologic physiologic phenomena. Postmortem examination has not resulted in a clear pathologic definition of this syndrome. Included in this group are patients with celiac disease, idiopathic

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steatorrhea, and sprue. Until these diseases are separated by etiologic or pathologic characteristics, the distinction remains a clinical one. They have been differentiated on the basis of age, place of residence, severity, response to treatment, reversibility of the disease, and other clinical manifestations[61,68,121].

Traditionally, the term sprue is best reserved for patients with an illness characterized by steatorrhea, diarrhea, stomatitis, glossitis, and megaloblastic anemia[149]. The effectiveness of different therapeutic agents in the syndrome[57,61,66,68] suggests diverse etiologies. The diagnosis of celiac disease is reserved for children with idiopathic steatorrhea. They may have additional clinical features related to the effects of malabsorption in young people. The familial coexistence of celiac disease and idiopathic steatorrhea[121] and the recent demonstration of their identical microscopic features on jejunal biopsy[134] support the concept that they are the same disease in patients of different ages. Similar microscopic features have been observed in sprue[26]. Remaining are a large group of patients with the disorder variously labeled as nontropical sprue or idiopathic steatorrhea. These adults may not appear seriously ill and may not have diarrhea. Adlersberg[1] proposed the term idiopathic or primary malabsorption syndrome to include celiac disease, idiopathic steatorrhea and nontropical sprue.

The modes of clinical expression of malabsorption are variable[28]. The presenting features may be related to inefficient absorption of fat, fat-soluble vitamins, water-soluble vitamins, electrolytes, minerals, or amino acids[155]. Symptoms may be clearly related to deficiency of one of these but may be nonspecific: weight loss, asthenia, changes in appetite or vague complaints[24,28]. The clinical features in a given patient are determined largely by the inefficient absorption of substances whose supply may be further limited by poor diet, deficient body stores, or increased demands. Thus, these patients may be asymptomatic when their requirements are met by dietary means. The defects in absorption for each substance appear to be partial rather than complete.

Thus, the clinical picture either closely resembles other better known disorders (for example, pernicious anemia) or is entirely nonspecific. Laboratory tests must be resorted to for demonstration of intestinal malabsorption and definitive diagnosis of these syndromes is possible only by these procedures.

ORAL ABSORPTION TESTS. Oral tolerance tests are the most widely used means of diagnosing intestinal malabsorption. Their popularity is based upon ease of performance, speed with which results are known, and fairly good correlation with the presence or absence of steatorrhea. Interest in these tests is reflected by the number of different ones devised and by the numerous publications that follow the introduction of a new test (I^{131}-labeled oils) or re-evaluation of older tests (xylose tolerance). Most procedures measure blood levels of the test substance after oral administration of an amount, which may or may not be calculated upon body weight. In many cases urinary or fecal excretion is also estimated.

No general agreement exists concerning the best technique for the performance of these tests. Some of the variables include: (1) amount of the test material to be given, (2) diurnal and other factors that may make time of day important, (3) activity of the subject during the test period, (4) previous nutritional state, (5) whether the patient should fast before and

during the test, and (6) gastric emptying time, which may be estimated by fluoroscopic or roentgenographic means.

The method of expressing results likewise varies. For example, results may be expressed as absolute blood values, percentage of the amount given or percentage of blood volume. Thus, each laboratory must establish a standard procedure and ranges of normal for each test. Abnormal response may be determined by various means: reliance upon the peak value of a curve that results from a multiple determination, absolute or percentage difference between fasting and peak values, and other characteristic features of the curve. Regardless of these variables, the conclusions drawn from a particular test performed by different investigators are usually in good agreement. In general, the correlation between the results of the more popular absorption tests and the presence or absence of steatorrhea is fair. Since it is imperfect, however the search for better oral absorption tests continues.

All tolerance curves express the interaction between absorption, metabolism, and storage of the test substance at the moment of sampling. Claims that degree of abnormality of an oral absorption test can be correlated with intensity of steatorrhea[20] have not been sustained. In fact, there is almost unanimous agreement to the contrary. The test substances are unpredictably affected by variation in gastric emptying, distribution in the blood, lymph, intra- and extracellular fluids, cellular utilization, storage, release, and excretion through the kidney and liver. The rates of absorption and release of the substance into the bloodstream are usually the limiting factors. Replicate tests may show fairly pronounced intra- and interindividual variation[91]. Hence, it is not surprising that only about 80% of any large group of patients with steatorrhea has a positive tolerance test with any given substance.

Tolerance curves are not too reliable for detection of mild malabsorption[155]. One might expect the reverse to be true, since time is an important factor in all tolerance tests and not of such importance in fat balance studies. However, at present the diagnosis of idiopathic malabsorption rests upon the demonstration of abnormal fat balance. A patient's general condition can vastly improve, and the tolerance curves can return to normal without any significant change in steatorrhea[52]. In this instance tolerance tests may be grossly misleading. The military personnel in Puerto Rico studied by Gardner[73], most of whom did not have steatorrhea but did have impaired tolerance curves, appear to be in a special category. Their clinical picture resembled mild sprue, but they were not anemic.

Functional hypermotility of the intestines that results in diarrhea may be confused with a malabsorption syndrome. Malins and French[109] have attributed diarrhea in their patients with diabetes mellitus and normal pancreatic exocrine function to rapid intestinal transit as a consequence of autonomic neuropathy. Only a few of these patients had steatorrhea, which was always mild, intermittent, and associated with severe diarrhea. It is in this situation that oral absorption tests may prove to be of great value, for hypermotility has been shown to increase the rate of absorption as determined by these tests. Cummins and Almy[40] demonstrated that parasympathomimetic drugs given to normal subjects resulted in higher tolerance curves for glucose and methionine. They postulated that this resulted from increased intestinal blood flow as a consequence of the increased motility. However a parasympathetic blocking

agent did not result in lower curves. Uyeyama and associates[154] demonstrated a threefold increase in galactose absorption in a patient with hypermotility. Mendeloff[113] has shown that sham feeding is nearly as effective as eating in enhancing serum vitamin A curves. He suggested that eating causes the lacteals to expel their contained vitamin A into the blood stream. Atropine was shown to delay but not to prevent the eating response. A higher intake of fat results in a greater percentage of absorption of fat in patients with pancreatic or idiopathic steatorrhea[15] but also usually produces diarrhea. Perhaps the enhanced absorption is due to increased motility related to the laxative effect of the undigested fat.

GLUCOSE TOLERANCE TEST. Measurement of blood glucose levels during fasting and at intervals of ½, 1, 2, and 3 hours after ingestion of 50 to 100 gm. of sugar is probably the best known and most widely employed test of absorption. A "flat" or depressed curve traditionally has been defined as a test in which there is less than 40 mg. per 100 ml. difference between the fasting and highest level. This is the usual type of curve associated with idiopathic malabsorption. In addition, Frazer and co-workers[62] have pointed out that the peak of the curve is delayed until after the first hour.

In only 6 of 94 patients with rather severe idiopathic malabsorption did Bossak and associates[24] obtain a normal glucose tolerance curve and these results seem to be representative[98]. Approximately 40% of normal persons may be expected to demonstrate a flat curve if a rise of less than 40 mg. per 100 ml. above the fasting level is the criterion used[73,117]. Consequently, an increase in excess of 25 mg. per 100 ml. has been suggested as the expected normal rise. Even with this value, 20% of normal persons exhibited a flat curve[73]. The depressed tolerance curve of normal subjects does not appear to be related to delayed gastric emptying and frequently is normal upon re-examination[153].

The glucose tolerance test has more inherent disadvantages than most oral absorption tests, since the liver, muscles, and endocrine glands affect glucose metabolism. A patient with steatorrhea and normal pancreatic exocrine function, as determined by the usual tests, may have a diabetic type of curve. Patients with apparent coexistent diabetes mellitus and idiopathic steatorrhea usually have a family history of diabetes, which assists in their differentiation from patients with chronic pancreatitis. In the absence of this family history, a diabetic curve in a patient with steatorrhea is strong presumptive evidence of pancreatic exocrine insufficiency. Herein lies the principal value of the glucose tolerance test in the malabsorption syndromes. Diabetic curves have been reported in 80% of patients with chronic pancreatitis[98]. Each of 10 patients studied by Gaddie and co-workers[72] with steatorrhea due to pancreatic exocrine insufficiency had a diabetic glucose tolerance curve, whereas a similar number of patients with idiopathic steatorrhea had flat glucose tolerance curves. Fructose offers no advantage over glucose as a test substance[58].

VITAMIN A TOLERANCE TEST. The vitamin A tolerance test is a time-honored test of intestinal absorption. Seventy-five thousand[113] to 180,000 μg.[24], or 10,000 international units per kg. of body weight, of an esterified form of vitamin A in an oily solution or 300,000 units of vitamin A as cod liver oil[76] is administered orally. Blood levels of the vitamin are measured at intervals extending to 9 hours, and in some lab-

oratories to 24 hours. Levels are enhanced by eating, so it is customary to have the subject consume a standard breakfast after ingesting the vitamin. Better results are said to follow administration of vitamin A before the test dose to insure adequate tissue stores[13]. Measurement of fecal loss of the vitamins apparently is more reliable than the blood levels, but this is tedious. Barnes and associates[13] observed a normal curve in a patient with steatorrhea, even though an excess of the test dose appeared in the feces. It is generally agreed that at least a fourfold rise over the fasting level should be present normally[113]. The blood level at 4 to 9 hours should be at least over 125 μg. per 100 ml.[76]. For comparison, a tolerance test with use of an aqueously dispersed form of vitamin A instead of the oil solution may be performed. Blood levels following administration of the aqueous solution are characteristically slightly higher in both normal subjects and patients with idiopathic steatorrhea[13]. Patients with pancreatic insufficiency theoretically might absorb the aqueous dispersion of vitamin A, yet fail to absorb the vitamin A in oil.

Many observers[3,13,76,107] have reported the value of the vitamin A tolerance curve as an indicator of the presence of steatorrhea. However, results are frequently inconsistent, and the test is influenced by other diseases[98,156]. The Mt. Sinai series of nearly 100 patients with idiopathic malabsorption appear to reflect general experience; 81% had a flat curve. Legerton and associates[107] obtained a good correlation between the vitamin A tolerance curve and the coefficient of fat absorption, but this has not been confirmed. Hillman and Becker[91] reported wide variations in replicate tests in the same individual and among different groups of subjects. Improvement in the general condition of the patient is poorly correlated with this test[4].

The I^{131}-labeled lipid tolerance curve appears to be exceptionally well correlated with the vitamin A tolerance curve[18]. This procedure is much easier to perform if facilities for counting radioactive materials are available, and it is likely to gain popularity at the expense of the vitamin A determination (see page 87).

Recently, interest has been aroused in the fasting serum carotene level as a screening procedure for the malabsorption syndromes[158]. A low level was found in 51 of 58 such patients. However, low serum values may reflect poor diet, liver disease, and such nonspecific factors as fever.

WATER DIURESIS TEST. Wollaeger and Scribner[160] in 1951 drew attention to the delayed excretion of water and nocturnal diuresis observed in patients with sprue. They found that absorption of water was delayed when water was taken during or after a meal and that nocturnal diuresis was abolished by fasting. It has since been shown by Taylor[151,152] that water diuresis in these patients is delayed even during fasting.

Whether absorption of water, which is thought to occur throughout the gastrointestinal tract, is a passive process secondary to the active translocation of electrolytes and organic solutes or is an active mechanism is unknown, but opinion seems to favor passive absorption[104]. That the diminished intestinal motility present in sprue may be an important factor in delayed water absorption is indicated by the work of Higgins and co-workers[89], who showed that drug-induced depression of intestinal motility also depresses the rate of absorption of water and sodium in normal subjects.

To perform the water diuresis test, hourly urinary volumes are measured

after oral administration of either one liter of water or an amount calculated upon body weight[151]. Normally, the given amount is accounted for in several hours urinary volume, whereas in idiopathic steatorrhea only a small percentage of the dose is recovered in the total 4 hours' volume[45]. The overnight volume may be compared with the volume excreted after the water load[45]; some hourly specimen should normally exceed the overnight volume.

Diminished diuresis is common to a wide variety of diseases: Addison's disease, renal disease, heart failure, and cirrhosis. Almost any illness may result in a positive test. Diez Rivas and associates[45] found only 1 of 18 patients with sprue who had a normal test, and in Cooke's[34] experience the test is commonly positive.

In general, the rate of water absorption in patients with sprue parallels their clinical condition[90], there being a significant correlation between delayed water diuresis and fat absorption[151]. However, no relation exists between fecal excretion of water and of fat[151]. Patients with pancreatic steatorrhea frequently have positive tests[151], as do those with pernicious anemia, in whom water diuresis tends to improve after therapy with vitamin B_{12}[150].

Thus, the water diuresis test yields valuable diagnostic information in intestinal malabsorption in that a normal result is decidedly unusual but an abnormal test is common to a wide variety of other pathologic states.

SERUM ELECTROLYTES. Electrolyte malabsorption is common in idiopathic steatorrhea even without diarrhea[34]. Higgins and associates[90] found the rate of radioactive sodium absorption more retarded than the rate of water. Cooke and co-workers[39] found a direct relation between fecal excretion of sodium and of water. Fecal loss of potassium paralleled that of water only in the presence of severe diarrhea. Excretion of potassium in the stool was unrelated to that of water or fat in the nondiarrheic state. Total body exchangeable potassium is diminished in steatorrhea[54]. Hence, even with normal serum electrolyte values hypokalemic nephropathy[130] and signs such as distention, ileus, and weakness may appear. According to Cooke[34], many of these manifestations subside after potassium therapy is instituted.

Normal serum electrolyte values are not uncommon in idiopathic steatorrhea. However, low serum levels, or clinical syndromes associated with potassium deficiency may be clarified by the demonstration of defective intestinal absorption.

Estimation of electrolytes in sweat[136] is a sensitive and reliable means of diagnosing fibrocystic disease of the pancreas. Excessive loss of sodium, chloride, and potassium in sweat is apparently due to an abnormality present in the sweat glands of these children. It is the most specific test yet devised for this disease[98] and has largely replaced such procedures as the measurement of urinary iodine after oral administration of lipiodol[145] and determination of ability of fecal enzymes to digest the gelatin film from a Roentgen-ray plate[139].

D-XYLOSE TOLERANCE TEST. The d-xylose absorption test is probably the most valuable of the available absorption tests. Xylose is neither absorbed against a concentration gradient nor significantly degraded by the body. Although it is probably actively absorbed in the small intestine, it is not phosphorylated in its passage through the mucosa. It is not metabolized by the liver but is partially destroyed by the tissues[59]. Urinary excretion is related to its concentration in plasma and is not affected by diuresis or exercise[46].

Twenty-five grams of the sugar is administered orally, and the amount present in the following 5-hour urinary volume is measured. The average amount recovered from normal subjects is 6 gm.; patients with idiopathic malabsorption usually excrete less than 1 gm.[59]. Blood levels may be determined at intervals, but are sufficiently variable to cause an overlap between normals and patients with poor absorption[17,76]. Normally 19 gm. of xylose is unaccounted for and, presumably, unabsorbed. Urinary excretion is diminished by ingestion of food during the performance of the test[17].

A good separation between normal and abnormal subjects has been found by all investigators thus far[17,76,98]. The test correlates well with changes in the clinical condition of the patient and is reasonably reproducible[17,25,57]. It has many obvious theoretic and practical advantages over the vitamin A, I^{131}-labeled oil, and glucose tolerance tests. Normal results have been reported in patients with pernicious anemia[88] and chronic pancreatitis[17].

More data are needed on large groups of patients to evaluate this test further. Thus far, d-xylose appears to possess more characteristics of an ideal oral absorption test than any test yet advocated.

AMINO ACID TOLERANCE TESTS. Several investigators have attempted to evaluate intestinal absorption by measuring blood amino acid nitrogen levels after oral administration of amino acids or a protein such as gelatin[5,7,40,154]. Patients with pancreatic insufficiency may have flat curves after administration of protein and normal curves when amino acids are given. Those with idiopathic malabsorption tend to have flat curves after administration of either substance. The test has not been widely evaluated in the diagnosis of intestinal malabsorption, and most investigators do not find the information gained to be commensurate with the difficulty of the chemical technique involved.

STARCH TOLERANCE TEST. Althausen and Uyeyama[6] suggested the starch tolerance test as an aid in the diagnosis of adult pancreatic insufficiency. This consists in comparing the blood sugar curve after oral administration of 100 gm. of starch with the glucose tolerance curve. They obtained an abnormal response in 87% of their patients with clinical pancreatic insufficiency.

Interpretation of the test is difficult, and the presence of diabetes makes it unreliable. It has, therefore, not gained wide acceptance. Kalser[98] described 2 patients who had abnormal starch tolerance tests, yet at laparotomy both had a normal pancreas and hepatitis.

RADIOACTIVE TRIOLEIN ABSORPTION. I^{131}-labeled triolein is one of the most recent and probably the most promising of the absorption tests. In the fasting state, 25 to 100 μc. of the test material is administered orally, together with a small amount of olive oil[112] or peanut oil[16]. Some investigators also use a few ml. of "tween 80" as an emulsifying agent and barium sulfate if gastric emptying time is to be estimated[16]. Beres and co-workers[18] give only milk and a carbonated beverage with the oil. Blood and fecal radioactivity may then be measured at intervals. Iodine is given for 3 or 4 days before the test to block thyroid uptake of radioactive iodine.

The appearance and disappearance of radioactivity in the blood stream has been shown to agree closely with that of chemically determined triglycerides[84]. However, only 20 to 35% of whole blood radioactivity is due to lipid bound I^{131} [18]. Normal subjects degrade 50 to 75% of the I^{131}-labeled oil in the first 24 hours[146]. This free iodine may result from metabolism of the

radioactive lipid before, during, or after absorption.

Due to this splitting off of the labeled iodine, some investigators count only the lipid fraction of serum[18], some measure whole serum[112], and others count whole blood[16,19]. The results may be expressed in terms of radioactivity corrected for an assumed blood volume[16] or as a simple percentage of the given dose[18]. Regardless of the variables, the curves obtained by these techniques are remarkably similar. Counting of whole blood will probably become the most generally used method.

The peak of the blood radioactivity curve normally occurs at 4, 5, or 6 hours. Berkovitz and Sklaroff[19] measured the blood level serially until the peak was reached and then discontinued the test. Reemstma[128] considered measurement of the 4-hour level alone adequate.

As yet, no investigator has reported real difficulty in separating the "flat" curve of malabsorption from the normal one. Characteristic and strikingly reproducible curves have been obtained by Baylin and associates[16], whereas others have not had as much success[112]. Ruffin[131] has explained his better results on the basis of routine roentgenographic or fluoroscopic correlation with gastric emptying and the maintenance of a fasting state in the patient.

Ruffin and co-workers[132] attributed flat curves to delayed gastric emptying or to malabsorption. He found that patients with disease of the colon and functional intestinal complaints invariably had normal curves. Mineral oil ingestion did not affect the tolerance test. Chinn and associates[30] did not find impaired absorption in older persons. In one series of patients with pancreatic disease, 90% had flat curves[143]. The test does not permit detection of early pancreatic disease[18].

I^{131} triolein tolerance correlates well with vitamin A tolerance[18] and, in all likelihood, will be shown to have similar limitations. The flat glucose tolerance curves occasionally seen in normal subjects cannot be attributed to delayed gastric emptying[153], and it may be erroneous to attribute flat triolein curves to this mechanism. The possibility of a normal tolerance curve in a patient with increased fecal excretion should be investigated, since such was found to be the case with vitamin A tolerance. Both false positive and negative results will probably be reported more frequently as the test gains wider usage, if blood levels are relied upon solely. Some investigators[112] already consider the determinations of blood curves alone to be inadequate.

Measurement of fecal radioactivity for 2 to 3 days after oral administration of the radioactive lipid has been shown to correlate well with blood curves[133], blood and stool levels being inversely related. Normally, less than 2% of the radioactivity is recovered in the stools. Fecal results are more similar to fecal fat studies[135] and will probably prove better for detection of mild steatorrhea, since total absorption and not absorption rate is measured. The urinary excretion data has not proved to be valuable by any investigator.

The I^{131} triolein is a simple tolerance test which is easy to interpret and relatively reproducible. It appears that it will supplant the vitamin A tolerance test in many instances. If facilities for counting radioactivity are available, the fecal counting procedure holds even greater promise and may well be the means by which steatorrhea can be detected with a high degree of accuracy. No large scale correlation of the fecal test with the coefficient of

fat absorption as determined by a balance study has yet been reported.

I^{131} OLEIC ACID ABSORPTION TEST. Additional value to the I^{131}-labeled triolein test has been given by the introduction of the I^{131}-labeled oleic acid oral absorption test. Since oleic acid does not require digestion before absorption, pancreatic malabsorption of triolein can be differentiated from other malabsorption. The procedure for the test and blood curves for the radioactive fatty acid are similar to those of triolein.

Patients with pancreatic insufficiency who have impaired triolein absorption almost invariably have normal fatty acid absorption[93]. Reemstma's[128] patients with "pure" pancreatic deficiency due to operation, obstruction of a duct or fibrosis, that is, those in whom steatorrhea would be expected, all had low triolein and normal oleic acid curves. Interestingly, patients with fibrocystic disease of the pancreas, like those with nonpancreatic steatorrheas, had relatively flat curves after administration of both substances.

I^{131} PROTEIN ABSORPTION TEST. I^{131}-labeled casein[30,106] or albumin mixed with gelatin[16] has been employed in a manner similar to that of triolein in the study of protein absorption. On the whole, the results have been fairly satisfactory. Its primary use in the malabsorption syndromes is in the study of pancreatic disease, as patients with idiopathic steatorrhea do not commonly have the degree of protein malabsorption that is associated with pancreatic disease[144], but the separation between different groups of subjects is not as good as that obtained with triolein. The advent of I^{131}-labeled oleic acid has provided what appears to be a much better test, and it seems likely that it will largely replace the protein test.

BUTTER FAT TOLERANCE TEST. Frazer and Stewart[64] performed blood chylomicron counts in healthy persons after ingestion of meals and described a characteristic curve that was obtained by plotting the values against time. This method has been used as a means of diagnosing intestinal malabsorption of fat.

Chylomicron counting is tedious, and the resulting curves are not reproducible. Accordingly, measurement of blood lipids[3,21] or of serum optical density[76,118] after oral ingestion of butter has been used. The resulting curves in patients with sprue tend to be flat[3,76], but Gardner[76] found the overlap with normals to be excessive.

ANEMIA. The presence of anemia has been underemphasized as a screening procedure in the diagnosis of idiopathic intestinal malabsorption. All 100 patients reported by Cooke and associates[38] were anemic. They regard a persistently normal blood count as strong evidence against idiopathic steatorrhea.

Frequently, the only clinical manifestation of malabsorption is megaloblastic anemia. It is difficult to differentiate this state from pernicious anemia and nutritional macrocytic anemia. Microbiologic estimation of serum vitamin B_{12} levels is of some value, but the procedure is not widely available. Low levels are found in pernicious anemia and nutritional macrocytic anemia and may be found in idiopathic malabsorption[114,119,120].

The frequency of apparent achlorhydria in patients with idiopathic malabsorption has led to the erroneous diagnosis of pernicious anemia. Kay[101] developed a procedure in which maximal output of gastric juice is consistently obtained by giving 0.04 mg. per kg. of histamine. Antihistaminic drugs are used to control the side effects of the large dose of histamine. Evidently, this dose of histamine stimu-

lates all the parietal cells maximally. By this method, true achlorhydria was found only in patients with pernicious anemia. This was confirmed by Card and co-workers[29] after they performed the test on 500 patients.

FOLIC ACID ABSORPTION. Microbiologic estimation of folic acid excreted in the urine after subcutaneous and oral doses of 5 mg. of folic acid has been used by Girdwood[77] to investigate intestinal absorption. Patients with sprue excrete a smaller amount of folic acid in the urine after administration of the oral dose. It may be necessary to load the tissues with folic acid before the test is performed. Girdwood[78] had no false positive results with this test; only 2 of 30 patients with sprue had a normal test. Of special interest are the 4 patients with normal fat balance but other signs typical of intestinal malabsorption and a positive differential urinary folic acid excretion test. Girdwood's test is the only one at our disposal for investigation of folic acid deficiency and appears to have many merits. However, his results need confirmation. The test will probably never be widely used because of the difficulties in measuring folic acid.

VITAMIN B_{12} ABSORPTION. The study of patients with megaloblastic anemia is greatly facilitated by investigating their ability to absorb radioactive vitamin B_{12}. Studies of this sort were first reported by Heinle and associates[87]. They gave vitamin B_{12} labeled with radioactive cobalt 60 to patients and detected poor absorption by increased radioactivity in the stools collected over a 7- to 10-day period. Absorption could be brought into normal range in patients with pernicious anemia by oral administration of intrinsic factor. Schilling[137] was able to detect absorption of radioactive B_{12} by counting urinary excretion. The urinary excretion could be demonstrated only if a large dose of nonradioactive vitamin B_{12} ("flushing dose") was given parenterally after the orally administered radioactive vitamin B_{12}. The massive excretion of nonradioactive vitamin B_{12} in the urine carried with it radioactive vitamin B_{12} in amounts that could be counted accurately. Glass and co-workers[81] were able to detect absorption and storage in the liver by counting externally over that organ. Hepatic counting could be reliably performed only after an interval of 5 to 7 days after oral administration of the tracer dose of radioactive B_{12}, that is, after the unabsorbed radioactivity was eliminated from the intestine in the stool. Recently, Glass and Boyd[80] reported an accelerated method of measurement of hepatic uptake of the cobalt 60, with use of castor oil and an enema to rid the intestines of unabsorbed radioactivity so that liver counting may be performed in 48 hours. Measurement of serum radioactivity[23,48] at intervals after oral ingestion of vitamin B_{12} Co^{60} has also been used successfully.

In all these methods, the amount of oral tracer dose of vitamin B_{12} Co^{60} is physiologic, that is, 0.5 to 2.0 μg. Glass[79] has demonstrated the sharp fall in the percentage absorbed when more than 1 μg. of B_{12} is administered, so approximately 0.5 μg. is the amount generally used[115,119].

Glass and Boyd[80], in a summary of the advantages and disadvantages of the various methods, pointed out that the results obtained with all these techniques are comparable. The Schilling 24-hour urinary excretion test[138] probably enjoys the widest popularity. It appears to be unusually reliable, rapid, and easy to perform and gives rather clear-cut results[80]. The amount of tracer oral dose has been modified, as has been noted. The 1000 μg "flushing dose" of nonradioactive vitamin B_{12}

may be given simultaneously with the oral dose[54,119,129] instead of 2 hours later, as originally used by Schilling. Miller and associates[115] administered the "flushing" injection 3 hours and again 24 hours after administration of the tracer dose and measured the 48-hour rather than the usual 24-hour urinary volume. They recovered a higher percentage of radioactivity by this means.

Results obtained by various modifications of Schilling's test do not differ in clinical interpretation. Many modifications are necessitated by the sensitivity of the radioactive counter that is available and the size of the aliquot of urine to be counted[53]. The urinary excretion test is invalid in the presence of severe renal insufficiency[115]. Many patients with acute infections demonstrate poor absorption on each of these tests[27,87,115]. Recent studies have demonstrated that prolonged administration of intrinsic factor-containing hematemics may depress the subsequent response to intrinsic factor in patients with pernicious anemia[140]. Since the major site of vitamin B_{12} absorption seems to be the lower ileum, these tests may prove useful in diagnosis of disease limited to that site[36].

With each method, patients with pernicious anemia fail to absorb significant amounts of radioactive vitamin B_{12}, but when a commercially obtainable intrinsic factor preparation is added, normal absorption results[48,80,81,87,137]. Normal absorption is present in patients with megaloblastic anemias associated with nutritional deficiency of vitamin B_{12}[125], pregnancy[56], fish tapeworm infestation[103], and cirrhosis[105].

Most patients with idiopathic malabsorption fail to absorb radioactive vitamin B_{12} both with and without the addition of intrinsic factor. This malabsorption pattern was demonstrated in 20 of 25 patients with idiopathic malabsorption reported by Oxenhorn and associates[119] and in 9 of 13 studied by Reisner and coworkers[129]. Clinical improvement in these patients is not consistently accompanied by better results of tests[71].

The results of tests in patients with malabsorption of vitamin B_{12} due to "blind loops" or "stasis" may or may not revert to normal after oral administration of an antibiotic for several days[10,47,83].

The radioactive vitamin B_{12} absorption tests are of great value in differentiating the various types of megaloblastic anemias. They are easy to perform and interpret and are valuable not only in patients who are in relapse but also in the diagnosis of vitamin B_{12} deficiency regardless of etiology. Their use as a screening procedure for presence of intestinal malabsorption of fat is limited[71].

IRON ABSORPTION. Although macrocytic normochromic anemia is the usual type of anemia which develops in intestinal malabsorption, microcytic, hypochromic anemia due to malabsorption of iron is not uncommon[11,55]. Moore[116] estimated that about 6 years is required for a previously normal adult male to become iron-deficient if the normal rate of excretion persists while no iron is absorbed. A patient with idiopathic steatorrhea may never absorb iron normally and may not have normal body stores of this element. Signs of iron deficiency may develop fairly soon after surgical procedures and other events that deplete body stores.

Patients with steatorrhea and anemia may develop macrocytosis for the first time after iron therapy has been prescribed for hypochromic anemia, or microcytosis may become evident after folic acid or vitamin B_{12} therapy. Of interest are those patients who present

as refractory cases of iron deficiency and are subsequently shown to have steatorrhea and malabsorption of iron[28,86]. Their iron deficiency is easily correctible by parenteral administration of iron.

Absorption of iron may be investigated by measuring radioactivity in blood or feces after radioactive iron is given orally[11] or by measuring levels of iron in the serum at intervals after oral administration of iron. Interpretation of these tests is difficult, apparently because of the small amount that is normally absorbed[96]. Many patients with sprue who are not demonstrably iron-deficient have flat blood curves[73], but there is much overlap with normals.

SECRETIN TEST. Collection of pancreatic secretions by means of a double-lumen tube placed near the ligament of Trietz after intravenous secretin stimulation remains the most specific and accurate test available for detection of pancreatic exocrine insufficiency[50]. The tube must be positioned carefully by fluoroscopic means. Either 1 unit per kg. of body weight or a standard dose of secretin is injected. The secretions may be examined for volume, bicarbonate, enzymes, biliary pigments, malignant cells, and parasites. If one enzyme is diminished usually all are diminished so that only amylase need be measured.

On the basis of different responses in volume, bicarbonate, and amylase, Dreiling[49] has differentiated two types of pancreatic defects. A quantitative diminution in volume with normal bicarbonate and enzyme concentration suggests ductal obstruction, whereas a qualitative change characterized by normal volume but less amylase and bicarbonate is observed in chronic pancreatitis. The specialized nature of this differentiation is apparent. Dreiling and Janowitz[50] have had great success in differentiating by the secretin test the normal response of patients with idiopathic steatorrhea from that of patients with pancreatic insufficiency. Three of 36 patients with sprue had an abnormal secretin test; all were considered to have pancreatic disease secondary to malabsorption. This is a logical consideration in view of the known pancreatic atrophy that accompanies poor nutritional states[43]. The secretin test may sometimes provide information that is difficult to interpret.

Measurement of serum amylase and blood coagulating factors after intravenous administration of secretin and other drugs has not proved helpful in the diagnosis of pancreatic insufficiency[50,51,148].

CALCIUM METABOLISM. Steatorrhea is frequently accompanied by diminished absorption of calcium, phosphorus, and vitamin D. Loss of calcium is thought to be due to malabsorption of calcium *per se*, only a small amount of fecal calcium being accounted for by the formation of calcium soaps and the loss of vitamin D[12,161]. Bile and fat are necessary for absorption of vitamin D. These losses usually result in a normal or low level of calcium and phosphorus in the serum, whereas the level of alkaline phosphatase in the blood is raised[85]. Both tetany and osteomalacia may result. Osteomalacia and tetany are not unusual manifestations of idiopathic steatorrhea[28]. About one-fourth of the patients at Mt. Sinai Hospital had tetany and one-half had roentgenographic manifestations of osteomalacia or osteoporosis[24]. Lowered levels of other electrolytes may enhance the appearance of tetany or, by themselves, produce it.

Recently, attention has been directed to the more unusual manifestations of disturbed calcium and phosphorus metabolism in idiopathic steatorrhea. These seem to be a consequence of

varying responses of the parathyroid hormone to lowered calcium and phosphorus levels. If the parathyroid response to excess fecal loss of calcium is normal, the phosphorus level in the serum should be low. Jackson and associates[95] studied a patient with steatorrhea, tetany increased serum phosphorus, normal response to infusion of calcium and no response to parathyroid extract or vitamin D. He called attention to similar patients reported in the literature, some of whom do respond to parathyroid extract[94]. None of these patients had the skeletal manifestations of pseudohypoparathyroidism. He considered steatorrhea with raised serum phosphorus levels to be uncommon but little noticed because tetany with low serum calcium can result with either a normal or high serum phosphorus.

Davies and associates[42] pointed out that patients with steatorrhea can demonstrate changes characteristic of both osteomalacia and hyperparathyroidism. They presented evidence to show that in some patients with idiopathic steatorrhea there develops hyperparathyroidism that responds to vitamin D; after prolonged malabsorption, parathyroid adenomas may develop. Dent[44] mentioned a patient with steatorrhea who had a low level of calcium in the serum but increased levels of phosphorus and alkaline phosphatase and osseous changes in the roentgenogram diagnostic of hyperparathyroidism. Vitamin D therapy corrected these.

ROENTGENOGRAPHIC FINDINGS. Radiologic examination of the small intestine may provide information of great importance. Snell and Camp[147] in 1934 observed dilation and smoothing of jejunal contour, with segmentation and clumping of barium, in 7 patients with idiopathic steatorrhea. They suggested that these changes were probably not specific. Pendergrass and associates[124] found that olive oil mixed with standard barium preparations produced a radiologic picture in normal subjects that might be confused with the pattern observed in steatorrhea. Frazer and co-workers[63] reported experiments indicating that some roentgenographic changes are indeed nonspecific and related to flocculation of barium. Gross clumping of barium was demonstrated when mixed with mucus *in vitro* and in segments of intestine obtained at necropsy. The degree of clumping was proportional to the quantity of mucus added. Pronounced segmentation of the barium was produced in normal subjects by intraduodenal administration of fatty acids. It was conjectured that the fatty acids may have produced their effect by stimulation of mucous secretion. Some confirmation of the role of fatty acids in producing the deficiency (segmentation) pattern may be found in the report of Lowe and associates[108]. They administered 30 to 60 gm. of corn oil with barium to children with pancreatic fibrosis and observed a normal pattern. When pancreatic enzymes were added, a clumped (deficiency) pattern was noted.

When nonflocculating barium[102] is used, the mucosa may be studied in greater detail[8,9]. The more fluid barium solution flows between the mucosal folds and demonstrates them to be enlarged. When the mucosal folds are prominent, a "stacked coil" or "ladder appearance"[67] may be seen.

Important information which can be evaluated in the light of many years experience can be obtained from radiologic studies of the small intestine with use of standard barium mixtures. Dilation of the mid and distal jejunum, one of the most important radiologic findings, was observed in 40 of 46 cases reported by Marshak and associates[111]. In 36 patients the dilation was twice normal or greater, which exceeded that

found in diseases associated with a clumped pattern, such as deficiency states, pellagra, nephrosis, cirrhosis, and hyperthyroidism. All 75 patients with malabsorption examined by Cooke and co-workers[38] with a flocculating barium emulsion showed varying degrees of the deficiency pattern.

Thickening of the mucosal folds with absence of the normal feathery or herringbone pattern was demonstrated in most of the patients reported by Adlersberg and co-workers[2] without resorting to nonflocculating barium. Segmentation of barium into large clumps with radiolucent intervals was commonly noted. The moulage sign was seen in 6 of their patients. This sign was described as "resembling a tube into which wax had been poured and allowed to harden[100]." Scattering, in which a snowflake appearance is given by small clumps of barium, is usually seen in combination with segmentation[110]. It seems likely that segmentation, moulage sign, and snowflaking may all be artifactitious, the result of greater or lesser degrees of clumping of barium and that this clumping may be related to a quantitative[67] or qualitative[75] mucous defect.

Inadequate emphasis has been placed on the value of small intestinal mucosal detail to the physician who does not have access to many of the diagnostic procedures mentioned in this review. Pancreatic steatorrhea and pernicious anemia present a normal feathery mucosal pattern[69] and, thus, may be differentiated from intestinal malabsorption. A flat plate of the abdomen should be taken before the barium studies are begun. Such a film may provide the physician with important evidence of pancreatitis by demonstrating calcification in the area of the pancreas[14,33] or may demonstrate osteoporosis and osteomalacia.

MUCOSAL BIOPSY. Wood and associates[162], in 1949, introduced a flexible vacuum gastric biopsy tube, which provided a safe and easy method for obtaining gastric biopsies. Joske and co-workers[97] found gross gastric mucosal abnormalities in every patient with pernicious anemia studied by this method. However, these abnormalities do not appear to be specific for pernicious anemia. Gardner[74], using the gastric brush technique, discovered abnormal gastric mucosal cells in patients with sprue (but without achlorhydria) similar to those present in pernicious anemia. Davidson and Markson[41] have shown that patients with iron deficiency also exhibit these abnormalities, especially when achlorhydria is present.

Pathologic changes in the small intestine in sprue have previously been minimized or reported as normal, probably because of the rapid appearance of autolysis at necropsy[122]. Shiner[142] has used the oral route to obtain duodenal and jejunal biopsies by means of a flexible biopsy tube. Small intestine mucosal atrophy with villous deformity has been found in biopsies obtained in this manner[92], similar to those found in biopsies obtained at laparotomy[122]. It is too early to determine whether or not these are constant pathologic changes in sprue, but thus far they have been found in every case of idiopathic steatorrhea and sprue studied[141]. Recently Sakula and Shiner[134] have reported identical pathologic changes in a child with celiac disease. Shiner[141] on biopsy of the intestinal mucosa of a patient with pancreatic steatorrhea, found it to be normal. Paulley and co-workers[123] reported a patient with postgastrectomy steatorrhea who had a normal jejunal biopsy. At necropsy, they discovered areas of normal mucosa and areas of atrophic mucosa. Their biopsy specimen was apparently obtained from one of the normal areas.

Intestinal biopsy holds great promise as a means of diagnosing sprue and idiopathic steatorrhea and may well provide the long sought firm basis for diagnosis. More information is needed as to microscopic findings in unrelated disease states, especially those associated with poor nutrition.

FECAL FAT ESTIMATION. The demonstration of steatorrhea is the cornerstone in the diagnosis of the malabsorption syndrome. Steatorrhea is generally considered to be a constant companion to poor absorption of other nutrients[57]. One might anticipate isolated failure to absorb a nutrient other than fat, and Girdwood[78] has described 4 patients with clinical evidence of sprue who had abnormal folic acid excretion tests yet did not have steatorrhea. Such cases need confirmation.

Perhaps we overlook bedside hints as to the presence of steatorrhea. Many experienced observers believe that the presence of steatorrhea may be strongly suspected on the basis of characteristic odor and appearance of the stool, its consistency when manipulated with an applicator, and whether the stools float in water[22].

The silvery gray color of the stool in steatorrhea is regarded as characteristic[61,66]. This color is thought not to be due to its increased fat content but rather to excessive reduction of biliary pigments to a colorless form by increased activity of intestinal organisms. This increased activity is conditioned by increased amounts of unabsorbed nutrients in the fecal stream. The color approaches normal when chlortetracycline is given orally.

Probably a more reliable indication of steatorrhea is microscopic evidence of increased fat in the stool. Cooke and associates[39] found such evidence in 85% of 100 patients. The technique is simple[155] and is probably of greatest value when done by one person who can establish in his own mind some standard of normal. Pratt[127] was one of the first investigators to express some dissatisfaction with the method when he reported a stool to be normal on microscopic study yet subsequent analyses showed the fat content to be considerably increased. This discrepancy has been amply confirmed[20,65,157].

Chemical analysis of the stool must be resorted to for precise information as to degree of steatorrhea. Results that express fat content as percentage of dry matter of a random stool are unsatisfactory. The percentage of dry matter in feces is not necessarily constant[37], and there are technical objections to drying feces[99].

The method of van de Kamer and co-workers[99], which estimates total fatty acid content of wet feces, has proven to be satisfactory and is simple and rapid. The method involves titration of fatty acids against dilute sodium hydroxide with thymol blue as an indicator. Occasionally, fecal chromogens interfere with the accuracy of the titration. A method similar to that of Cooke and associates[37], in which the fatty acids are isolated by extraction and are then weighed, has certain advantages. Volatile fatty acids are lost by this method, but this should be of little consequence unless the dietary fat is mainly of dairy origin.

Regardless of the method used for estimating fat in the stools, a balance technique must be employed[70,121], in which the diet is of known fat content and the collections are adequate. Carmine markers are commonly used to indicate the beginning and end of the balance period. Greater accuracy may be anticipated from balance studies of greater than 4 days[60,159]. However, one can make a secure diagnosis of steatorrhea if the patient fails to absorb 95% of the dietary fat during the 4-day balance period[121].

Procedures that estimate both fatty acids and triglycerides have not gained much popularity in differentiation of pancreatic from idiopathic steatorrhea. The patient's response to a potent pancreatic enzyme preparation is of great help in this differentiation, but 12-day balance periods must be employed to assess accurately changes under therapy[60,155].

Steatorrhea, which may be shown not to be due to pancreatic or other causes of secondary malabsorption, is diagnostic of a primary malabsorption syndrome. Steatorrhea is considered by some to be rare in ulcerative colitis, even when secondary ileal involvement exists[35], although there is lack of agreement on this point[126]. Similarly, patients with hepatic disease and without jaundice or biliary obstruction do not have steatorrhea[35]. The only practical source of error would be in diminished absorption associated with acute infections[65] or severe malnutrition[37,82]. Fat absorption returns to normal after correction of these factors.

Discussion. The clinician's problem is twofold: (1) to determine the presence of gastrointestinal malabsorption and (2) to uncover its cause. Since fat appears to be the sole substance, absorption of which is constantly impaired in the malabsorption syndromes, the demonstration of steatorrhea is the ideal method of diagnosis. Lack of facilities for determination of fecal fat leads to the employment of other procedures, which are used with varying degrees of success.

A reliable history and physical examination are essential to disclose past surgical procedures which may have produced external fistulae or shunts or may have removed regions of the gastrointestinal tract important to absorption. Similarly, obstructive jaundice and severe hepatic disease may be dismissed. Patients who have a congenital insufficiency of lipase or bile salts resulting in steatorrhea without associated jaundice must be rare. One may search for evidence of disorders in which intestinal involvement is a part of the pathologic process, such as lymphoma, carcinoma, Whipple's disease, amyloidosis, scleroderma, and tuberculosis. Routine studies, such as the blood count and the roentgenogram of the chest may help exclude some of these disorders. In addition, the film may reveal osteomalacia with spontaneous fractures.

The blood count, if persistently normal, would tend to rule out primary intestinal malabsorption. An important anemia is not likely to be seen in pancreatic insufficiency and in addition, weight loss is not common in the absence of overt diabetes[70]. Macrocytic anemia is an indication for gastric analysis. If achlorhydria exists, even after an augmented histamine test, pernicious anemia is the most likely diagnosis. A Schilling test may be expected to give confirmatory information and appears to be the most valuable single procedure in the investigation of macrocytic anemias. If a patient with megaloblastic anemia has a normal Schilling test, one can only speculate as to the role of malabsorption of folic acid in the genesis of the anemia unless studies of folic acid absorption are available.

A patient whose microcytic hypochromic anemia has been demonstrated by repeated study of the stool and gastrointestinal tract, not to be due to chronic blood loss and who fails to respond to oral iron therapy may fail to absorb iron normally. Further studies for detection of steatorrhea are indicated.

The physician may next request radiologic studies of the gastrointestinal

tract. Unsuspected osteomalacia or calcification of the pancreas may be discovered on the preliminary film. The barium study may reveal intestinal diverticulae, stenosis, or internal fistulae of the small intestine. The small intestinal films may show a deficiency pattern with clumping of barium to a varying degree, a sign that must be regarded as nonspecific. If clumping is present to such a degree that mucosal detail is not obtained, a study of the small intestine with nonflocculating barium should be secured. Evidence of involvement of the small intestine by known pathologic process, especially regional enteritis, should be sought at this time and is detected with fair accuracy. A normal study is anticipated in the presence of pancreatic steatorrhea when nonflocculating barium is used.

At this stage of the examination, tolerance tests may be of value. A diabetic glucose tolerance in a patient without a family history of diabetes who is suspected of having steatorrhea is strong evidence to proceed with studies that are not commonly available, that is, the secretin test, determination of the presence and degree of steatorrhea by the fat balance technique, or studies that compare absorption of radioactive-labeled triglyceride and fatty acids. These tests, if properly performed, may give diagnostic information.

An abnormal xylose tolerance test suggests intestinal malabsorption, and normal results are anticipated in patients with pancreatic insufficiency. Vitamin A tolerance and starch tolerance curves do not yield diagnostic information, although they may be of some assistance. Alterations in serum electrolytes, although important, are not diagnostic.

Biopsy of the small intestine by the oral route is a recent procedure which holds promise in the diagnosis of intestinal malabsorption. However, its use at this time is limited by the lack of availability and general experience both in performance of the biopsy and in its interpretation.

The only certain method of excluding the diagnosis of idiopathic steatorrhea is demonstration of normal fat absorption by a balance technique. The van de Kamer method of estimating fecal fat has the greatest number of advantages and should be more widely used in the future. Determination of fecal radioactivity after oral administration of I^{131}-labeled oils may prove to be as satisfactory in demonstrating steatorrhea as a fat balance technique but insufficient data are available for final evaluation of this procedure.

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SUMMARIO IN INTERLINGUA

(See page 32 for original article)

Observationes Relative A Amanozine (W–1191–2), Un Composito Triazinic De Virtute Diuretic

Amanozine (W–1191–2) es un diuretico triazinic. Illo es nonmercurial, es administrate per via oral, e age como potente "diuretico de aqua." Illo causa solmente un leve augmento del excretion de natrium.

Post chronic administrationes oral de amanozina, studios laboratorial resulta in le constatation de significative sed reversibile grados de depression del hemodynamica renal, in simultaneitate con retention de nitrogeno e de phosphato de grados equal a illos notate post therapia chronic con le analogo chlorazanil. Le uso clinic de amanozina require multe attention. Isto suggere que su utilitate es restringite a cursos de oral therapia diuretic a curte vista.

Le mechanismo del action de amanozina, reflectite in le configuration del excretion de electrolytos, simula le effecto de su analogo chlorazanil. Iste duo compositos differe in le mechanismo de lor action ab organo-mercuriales, ab inhibitores de anhydrase carbonic, e ab chlorothiazido. Illos pare representar un quarte modo de comportamento diuretic.

NEUROLOGY AND PSYCHIATRY

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PSYCHOPHYSIOLOGICAL FACTORS IN DISORDERS OF THE COLON

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THE differentiation between organic and emotional causes of dysfunctions of the colon is not an easy task. Not only do similar symptoms (pain, constipation, diarrhea) occur in both organic disorders and in disorders associated with emotional maladaptations, but psychological reactions to disturbances of function further increase the symptoms of the disorder. Many different emotional states and conflicts can influence the function of the colon, and there are multiple physiological mechanisms through which these emotions can be mediated. Past variations in individual experiences also lead to vagaries of psychophysiological responses within a single individual as well as in comparisons between individuals. Further confusion arises when emotional significance is secondarily ascribed to symptoms and such secondary elaborations are misinterpreted to represent etiologically significant affects or emotional states. Because of these complex interrelationships, any discussion of emotional factors in disorders of the colon must cover both the psychological significance and the physiological mechanisms of colonic function. Increasing emphasis is found in the literature on this need to correlate emotional factors with the physiology of the system involved[6,16,20,26].

The major functions of the colon include reabsorption (principally of water), secretion, storage of feces, and elimination of feces[4,5,15,16]. Reabsorption and secretion takes place primarily in the right half with storage and elimination occurring principally in the left half of the colon[5].

Embryologically, the colon develops from both the mid-gut and from the hind-gut and receives parasympathetic innervation from the vagus and from the sacral parasympathetics[5]. Sympathetic innervation is derived from the thoraco-lumbar chain. In general, sympathetic stimulation results in relaxation of the colon with contraction of the sphincters, whereas parasympathetic stimulation increases motility with relaxation of sphincters. However, some motility occurs without relaxation of the sphincters, and there is some lack of clarification as to which activi-

ties are intrinsically controlled and which are predominantly under central nervous system control[15].

Szasz[25,27] cites the frequent incidence of diarrhea following vagotomy as evidence that the vagal and sacral components of parasympathetic innervation have reciprocal rather than concomitant actions. However, studies by Grace, Wolf and Wolff[16] seem to indicate that vagotomy has little influence upon colonic function. These authors point out that the emotional reaction to the operation may contribute to postoperative changes in function.

An additional parodoxical reaction occurs with some intense emotional states such as fear. Involuntary emptying of the colon associated with such emotions would seem to indicate that the sacral parasympathetics at times discharge simultaneously with the sympathetics[14].

The principal intrinsic and reflex motility patterns of the colon include[4,5,15,16]: the gastrocolic reflex which results in reflex evacuation of the colon with filling of the stomach (though it may also be associated with the psychic phase of digestion or anticipation of eating); segmenting movements, antiperistalsis, and pendular movements which promote reabsorption from the right half of the colon; peristaltic movements which transport the contents from the right side to the left side for storage; the defecation reflex which initiates evacuation as the contents reach the rectum; and reflex inhibition of the colon when there is contraction of the external anal sphincter[4,16].

During the processes of personality development and social adaptation with growth, many of these physiological functions become associated with emotional states of the individual. The gastrocolic reflex is active during infancy and childhood[24], and evacuation following filling of the stomach may give rise to childhood ideas that the abdomen is a hollow cavity. Such attitudes may later become unconscious but remain as components of "fantasies of function" within the adult[20]; and the concept of a hollow cavity may contribute to the development of childhood misconceptions concerning other "intra-abdominal" processes of impregnation, pregnancy, and parturition. Szasz[25] suggests that lack of oral gratification, based on infantile oral frustrations, may inhibit the function of the gastrocolic reflex and result in constipation. Lienteric or reflex diarrhea is described in adults following meals[12] and can be assumed to result from an over-active gastrocolic reflex. Mass peristalsis has been described in association with the sight or smell of savory food[4], so that gratification of hunger does not, in all cases, seem to be associated with this reflex. Though there is still need for further understanding of these psychophysiological interactions, it can be seen that certain sequelae of different oral tensions may be expressed physiologically through constipation or diarrhea[25].

Colonic function is one of the first functions around which the individual experiences discipline toward socially adaptive behavior through the procedure of toilet training[19]. Toilet training is essentially an inhibitive function, in that the child is taught when not to defecate, rather than when to do so[19,24]. The reflex inhibition of the colon through contraction of the external anal sphincter becomes an important component of this social adaptation, and training can probably best be undertaken after the child is able voluntarily to initiate this reflex inhibition. With toilet training, certain sensitive attitudes develop having to do with shame, modesty, disgust, humiliation, dirtiness, contempt, and other judgmental and

rejecting concepts[19]. Such attitudes and affects may become unconsciously related to autonomic functions of the colon.

Because colonic function is one of the early functions through which the individual can express independence of action, attitudes of obstinacy, withholding, and stubborness may develop. Parental reactions to the child and the process of toilet training have much to do with the development of such attitudes. However, such interaction between parent and child may initiate life-long character traits within the individual.

Freud[13] first described adults with character traits of orderliness, obstinacy, and parsimony associated with preoccupation with bowel function and with chronic constipation. Further description of such associations between emotional states and function are found in papers by Jones[17], Abraham[1], and Ferenczi[11]. A psychophysiological explanation is that unconscious fears of soiling, reactions against parental demands, and wishes to retain bodily productions result in chronic contraction of the external anal sphincter with reflex inhibition of colonic motility.

Most colonic functions are autonomic, and symptoms or disturbances of these functions, therefore, do not express any particular psychological meaning but rather are interpreted as vegetative or organ neuroses[2,25]. However, the external anal sphincter is under voluntary control and the symptoms of constipation, related to its contraction and the reflex inhibition of the colon, can more or less directly express unconscious conflicts. Of course, organic, irritative lesions leading to pain and spasm of the sphincter will also result in constipation on this basis. Rectal fissures and external hemorrhoids resulting from the passage of dry, hard fecal masses further add to the problem of constipation.

Jones[17] points out that defecation is a pleasurable experience and that the child may retain feces in order to prolong this pleasure or anticipation of the pleasurable relief. This pleasurable component contributes to the development of sexual overtones to colonic function[19], many of which may be autoerotic or homosexual in nature. Prohibitions against such erotic gratifications may further contribute to the reflex inhibition of the colonic motility with resultant constipation. Similarly withholding, itself, may take on pleasurable aspects[2]. Such attitudes toward colonic function frequently become displaced to other activities, resulting in the development of the previously described character traits.

In addition to the reflex inhibition or activation of colonic function, imbalance of sympathetic-parasympathetic innervation can also lead to disorders of function. Thus parasympathetic underactivity or sympathetic overactivity leads to constipation, whereas the opposite imbalance results in diarrhea. Hirschsprung's disease is an illustration of the first type of imbalance[4]. Without implying psychogenic etiology of this disorder, it is possiible to postulate that similar but less severe disturbances may be due to chronic sympathetic overactivity related to the emotional state of the individual.

Grace, Wolf and Wolff[16] note that the relaxed colon is associated with constipation, whereas increased motility is associated with diarrhea. However, a clinical syndrome of "spastic constipation" is described in which pain and spasm are associated with constipation. The use of laxatives, cathartics, and enemas in the constipated individual may contribute to the pain and spasm[23]. Superimposed upon the con-

stipation, and adhered to because of intense preoccupation with bowel function, such measures serve to complicate the evaluation of contributing causes. Excessive swallowing of air in states of emotional tension similarly contributes to these symptoms.

Other mechanisms of pain and spasm may be related to the dual parasympathetic innervation, vagal and sacral, of the colon. Reabsorptive and secretory functions occur principally in the right colon[5] and are augmented through the segmenting, pendular, and antiperistaltic motility of the colon. Overactivity of this motility, related to vagal hyperactivity, undoubtedly results in excessive dehydration of contents as well as in symptoms of pain and spasm. According to Szasz[25], this vagal activity also results in decreased sacral parasympathetic activity, with inhibition of emptying, thus further contributing to the constipation.

Sacral parasympathetics, on the other hand, innervate the eliminative functions of the colon[14], and overactivity of this component of the parasympathetic system is presumed to result in diarrhea. As previously noted, this system may discharge concomitantly with the sympathetics during certain intense emotional states[14]. Further, the sacral parasympathetics supply the sexual organs, innervating those physiological changes associated with sexual arousal. Prohibitions and inhibitions of sexual functions frequently seem clinically related to the development of diarrhea. Additional studies are required to elucidate the psychophysiological interactions of this component of the autonomic nervous system. Similarly, more information is needed concerning the role of sympathetic underactivity in the development of diarrhea or other disorders of colonic function.

Many difficulties are encountered in relating emotional states to the activities of the sympathetic and parasympathetic system. Certain of the "emergency responses" have been outlined by Cannon and other workers using similar techniques[4,14,15]. However, other emotions seem related to disturbances of function. Thus, though depression and paranoid ideas have been related to constipation[2], little is known concerning the psychophysiological mechanisms involved in the somatic expression of these emotional states. Certain theories such as the "vegetative retreat" of Alexander[2], "regressive innervation" of Szasz[26], and "psychophysiological regression" of Margolin[20] are all directed toward further understanding of these interactions. Engel[6] emphasizes the need for further study of the psychophysiology of such emotions as grief and depression as well as responses to events such as separation; and he points out that our present theories of psychophysiological responses may prove inadequate to explain the phenomena encountered in such studies.

These difficulties are well illustrated by studies of ulcerative colitis[2,3,7,8,9,10,16,18,22]. Engel[7] has given a complete description of the pathological process and a review of different theories of etiology[8]. Though space will not permit an extensive discussion of this disorder some mention should be made of the difficulties which this disorder presents in terms of understanding the psychophysiological relationships. Many factors have been suggested in the etiology, including toxins, allergens, bacteria, and chronic parasympathetic overactivity. It is recognized that constitutional and other unknown organic factors may influence the occurrence and progress of the disorder. Though psychic conflicts have long been recognized as having significance in this disorder, the mechanisms of mediation

of these psychological factors is unclear.

Engel[9] notes premorbid personality defects of long standing with characteristic dependent and restricted relationships with people and failure to achieve full heterosexual development. He also finds consistent psychopathology in the mothers of his patients. According to these studies, onset and relapses were found to occur when separations or threats of separations with key people were reacted to with helplessness, despair, and hopelessness. Where these separations or threats were not responded to by such affects, other neurotic, psychotic, or psychosomatic reactions were observed[9,10]. Engel stresses the mother-child relationship in determining the vulnerability of separation and relates the tissue reactions of ulcerative colitis to biological changes occurring consequent to traumatic separations[9]. He also suggests that such biological changes may alter relationships in the colon so that it becomes sensitive to its own flora as to pathogens or that the vascular system becomes responsive or reactive to previously harmless chemical substances.

In this connection, little is known of the developmental physiology of the colon or of other systems of the body. It is known that there are changes in rates of growth of different organ systems during maturational processes, and there are some indications that similar variations occur in other physiological activities. Margolin[20], in his discussion of psychophysiological regression, points out that greater fluctuations of the internal environment are tolerated in the infant than in the adult. The timing of such physiological changes in relation to emotionally traumatic events may have some significance in the type of reactions or in the system involved in psychophysiological reactions to similar emotional events in the adult. Longitudinal studies of variations in physiological activity during maturation, and correlation of these variations in function with events or processes of psychological maturation may shed some light upon the development of pathophysiological reactions to emotional stresses within the adult. Such studies should not be confined to the psychophysiology of the autonomic nervous system but should include physiological variations of other humoral and hormonal factors.

This multiplicity of both emotional and physiological factors associated with colonic function has certain therapeutic implications. Because of the complex interactions, it is frequently difficult to separate the organic from emotional influences, and diagnostic studies must include thorough evaluation of both the emotional and physical state of the individual. Individual variations in reactions to psychological factors must also be considered. Many facets of the therapeutic management of psychophysiological disorders have recently been discussed by Margolin[19,21].

In summary, some of the major physiological mechanisms of colonic function have been described with an attempt to discuss the influence of emotional factors upon these functions and resultant pathophysiological responses. Many of the physiological functions as well as the psychological states deserve more detailed discussion. Some areas for further investigation have been touched upon with emphasis upon the need for correlative studies of both physiological and emotional influences.

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MYASTHENIA GRAVIS: REVIEW OF RECENT ADVANCES WITH REPORT OF CASES SEEN AT SINAI HOSPITAL OF DETROIT

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THE sum total of knowledge concerning myasthenia gravis has been significantly increased over the past 10 years. It is the intent of this communication to present a review of current concepts relating to diagnosis, pathophysiology, pathology, and treatment of this disease, illustrating some of these with summaries of cases hospitalized at Sinai Hospital, Detroit.

Case Reports. CASE 1 (G.A.). This 14-year-old colored female was admitted for diagnostic study. There was a history of ptosis of both eyelids and occasional diplopia of 8 years' duration. Shortly before hospitalization a physician had administered Prostigmin intramuscularly and noted marked improvement of the bilateral ptosis. She was given Prostigmin orally. Two weeks prior to admission to Sinai Hospital on January 8, 1955, all medication was discontinued. Examination at the time of hospitalization revealed "slight drooping of both upper eyelids, more marked on the right." Hemoglobin, white blood count and differential were normal. Examination of the urine revealed no abnormalities. The Mazzini test was negative. Pulse and respiration were normal. There was no disturbance in speech and no difficulty in mastication or swallowing. Quinine sulfate in a dosage of 5 grains every 3 hours was administered, beginning on January 12, 1955, and ending on January 15, 1955. This did not result in aggravation of symptoms. She was discharged on January 15, 1955, with a provisional diagnosis of myasthenia gravis.

Subsequent course. Contact was not maintained after her discharge and no information as to her subsequent course is available.

CASE 2 (J.P.). This 20-year-old unmarried colored female was admitted to Sinai Hospital on May 31, 1955, complaining of generalized muscular weakness and diplopia of 4 weeks' duration. Examination showed a weakness of the left lateral rectus muscle. The patient was a known myasthenic, her illness having begun in 1952, approximately 3 months after she had given birth to a normal healthy full-term infant, when she began to complain of ease of fatigue, generalized muscular weakness, difficulty in swallowing and in holding her head erect. She responded well to treatment with 15 mg. of Prostigmin and ¾ grain of ephedrine sulfate four times daily. She had no further difficulty until the onset of diplopia and weakness 4 weeks prior to this hospital admission. A chest roentgenogram was reported as showing no abnormalities. However, barium swallow was not done. Basal metabolic rates were reported as −24 and −29% on two successive tests. Serum cholesterol was 210 mg. per 100 ml. Mazzini test was negative. Hemoglobin determination was within normal limits and there was no sickling of the red blood cells. The white blood cell count was

10,500 per c.mm. and urinalysis was reported as showing "bacteria and a few WBC." Dosages of Prostigmin and ephedrine were increased to five times daily and improvement of visual symptoms and weakness occurred within 48 hours. The patient was also started on ¼ grain of Proloid daily, beginning on June 6, 1955. She was discharged on June 10, 1955.

Subsequent course. She has continued to do well on the dosage of medication that she was taking at the time of hospital discharge.

CASE 3 (N.K.). This 17-year-old white female was admitted to Sinai Hospital on March 10, 1956, because of generalized weakness. Her mother stated that she had been a "weak baby." She had difficulty in sucking on the bottle and when the holes in the nipple were enlarged she choked easily. She did not begin to walk until 16 months of age. During childhood she was unable to keep up with other children her age in playing and running. She had been seen over the years by several doctors and, although a definite diagnosis had not been established, the possibilities of muscular dystrophy and amyotonia congenita were considered.

Recently she had begun to have increasing weakness of the eyelids and of the voice and difficulty in swallowing. This seemed to get worse toward the end of the day. She was given a test dose of Prostigmin intramuscularly in a physician's office prior to this hospitalization and dramatic improvement was noted.

During hospitalization she was given a barium swallow and observed fluoroscopically. This was reported as showing "vallecular and pyriform sinus retention without evidence of mass or other abnormalities in this region. After several attempts at swallowing they were both emptied." This was diagnosed as showing "neuromuscular disease of the pharynx with vallecular and pyriform sinus retention, compatible with myasthenia gravis or pseudobulbar palsy." The basal metabolic rate was −2%. Blood count and urine examination revealed no abnormalities. Electromyography was done and reported as follows: "Electromyogram of right deltoid, quadriceps, anterior tibial, gastrocnemius and hamstrings revealed no myopathic or denervation activity."

Treatment was started with Mestinon on March 12, 1956, beginning with a dosage of 30 mg. four times daily and progressively increased to 90 mg. four times daily on March 15, 1956. There was progressive improvement and the patient was discharged on March 17, 1956.

Subsequent course. She has continued to do well on the same dosage of Mestinon as that given at the time of discharge from Sinai Hospital.

CASE 4 (M.L.). This 66-year-old white male was admitted to Sinai Hospital at 10:30 p.m. on March 21, 1957. The admitting diagnoses were: (1) Incarcerated left inguinal hernia; (2) myasthenia gravis; (3) chronic bronchitis; (4) pulmonary emphysema.

The diagnosis of myasthenia gravis had been established in 1955, when he had complained of bilateral ptosis of the eyelids and generalized weakness. There was favorable response to a Prostigmin test and improvement was maintained thereafter on oral medication with 12.5 mg. of Mytelase four times daily. The incarcerated hernia was reduced nonsurgically shortly after hospitalization, and since the patient refused surgical repair he was discharged on the following morning.

Subsequent course. He has continued to control the myasthenia gravis manifestations with 12.5 mg. of Mytelase daily.

CASE 5 (M.R.). This 39-year-old white female, who had been hospitalized on several previous occasions for cholecystectomy, choledocholithotomy and for treatment of wound abscesses, was admitted to Sinai Hospital for the third time on December 4, 1955, complaining of faintness and vomiting, weakness, bilateral ptosis and slurred speech. A diagnosis of myasthenia gravis had been established by her doctor several months prior to this hospital admission. The diagnosis was based upon prompt alleviation of weakness, slurred speech and bilateral ptosis following oral administration of Prostigmin. Improvement had been maintained on continued oral medication until just prior to this hospitalization. In addition she had been treated with anticholinergic medication for relief of recurrent right upper quadrant pain, believed to be due to biliary ductal disease. The presenting manifestations resulting in this hospitalization had occurred shortly after administration of Demerol for relief of pain, and the possibility of Demerol sensitivity was considered as a cause of the faintness, nausea and weakness.

During this hospitalization an intravenous cholangiogram revealed hepatic duct enlargement, but no stones were visualized in the ductal system. Injection of Prostigmin, in addition to oral medication, was required for relief of marked ptosis and generalized weak-

ness. Oral glucose tolerance test was normal. Serum lipase and amylase determinations, as well as alkaline phosphatase determinations, were within normal range. Bromsulphalein retention was 6% in 45 minutes, and the basal metabolic rate was +5%. Roentgenogram of the barium filled colon and films of the chest revealed no abnormalities. Twenty-four hour urinary 17-ketosteroids excretion was within normal range.

During this hospitalization the dosage of anticholinergic medication (Probanthine) was progressively reduced and the dosage of Prostigmin progressively increased. Probanthine was discontinued on December 7, 1955. She was discharged on December 14, 1955, on oral Prostigmin in a dosage of 7.5 mg. four times daily. She was improved.

Fourth Sinai Hospital admission. She was re-admitted on May 13, 1956, because of severe recurring abdominal pain. Shortly after her previous hospital discharge on December 14, 1955, a diagnosis of thyrotoxicosis had been established on the basis of clinical manifestations and the results of I-131 uptake studies, as well as plasma I-131 determination. She was treated first with Tapazole and subsequently with I-131. At the time of this admission there was severe abdominal pain, frequent diarrhea and increased generalized weakness. The basal metabolic rate at this time was −25%. More frequent administration of oral Prostigmin was required, as well as frequent supplementary parenteral administration. Intravenous cholangiography at this time revealed the presence of a common duct stone. Operation for its removal, followed by insertion of a T-tube, was performed on May 29, 1956. Blood transfusion had been given preoperatively on May 28, 1956. During the operation and in the immediate postoperative period Prostigmin was administered parenterally. On June 1, 1956, she was able to take oral medication. She was discharged as improved on June 25, 1956.

Fifth Sinai Hospital admission. She was re-admitted on August 25, 1956, with a diagnosis of homologous serum jaundice. Treatment with oral cortisone was started on August 29, 1956, in dosage of 25 mg. every 6 hours. On September 7, 1956, the dosage of cortisone was reduced to 12.5 mg. every 6 hours. Because of increasing muscle weakness, he dosage of Prostigmin was increased to approximately twice the pre-hospitalization requirements. Prostigmin was left at the patient's bedside to be taken when necessary for weakness. On September 25, 1956, the dosage of cortisone was reduced to 5 mg. every 6 hours and instructions were left for the patient to be awakened daily at 2:00 a.m. for oral administration of 7.5 mg. of Prostigmin. During the next 3 days frequent subcutaneous administration of Prostigmin was necessary because of marked respiratory difficulty. The dosage of cortisone was meanwhile progressively reduced and discontinued on September 27, 1956. On October 2, 1956, there was less respiratory difficulty but more marked ptosis was noted. On October 12, 1956, Prostigmin was discontinued and treatment with Mytelase started. This was begun at a dosage of 12.5 mg. five times daily and progressively increased. She was discharged on November 4, 1956, with good control of the myasthenic manifestations. She was then taking 7.5 mg. of Prostigmin in the morning upon arising and 37.5 mg. of Mytelase at 9:00 a.m., 1:00 p.m., 5:00 p.m. and 10:00 p.m.

Subsequent course. She has continued to have periodic fluctuations in the severity of myasthenic manifestations requiring periodic adjustment of medication dosage. Fairly good control has been maintained with Mytelase.

CASE 6 (M.K.). This 43-year-old white female, known myasthenia gravis patient, was admitted to Sinai Hospital on August 26, 1955. The diagnosis had been established 6 weeks earlier when she had complained of profound muscular weakness, slurred speech, difficulty in swallowing, and bilateral ptosis. To some degree these symptoms had been present for approximately 18 months and had been getting more severe. Examination revealed no muscle atrophy and no reflex or sensory abnormalities. Response to the Prostigmin test was dramatic. Retention of swallowed barium in the valleculae and pyriform sinuses was demonstrated, and the retention was promptly evacuated after subcutaneous administration of Prostigmin. There was no roentgenographic evidence of thymic enlargement. Blood count and urinalysis revealed no abnormalities, and the basal metabolic rate was within range of normal. Response to oral Prostigmin was satisfactory during the first week of treatment, but requirements for medication thereafter rapidly increased. Three weeks after the diagnosis had been established, it was necessary for her to take 45 mg. of Prostigmin every 3 hours around the clock to permit deglutition of food and to prevent marked respiratory distress. At the time of this Sinai Hospital admission, a mild upper respiratory infection had aggravated the myasthenic manifestations. It became necessary to administer 60 mg. of Pro-

stigmin orally every 2 hours around the clock, as well as 1 mg. subcutaneously before each meal. On September 2, 1955, 37.5 mg. of WIN 8077 (now known as Mytelase) was substituted for the 2:00 p.m. and 6:00 p.m. doses of Prostigmin. On September 3, 1955, oral and parenteral Prostigmin were discontinued and 37.5 mg. of WIN 8077 (Mytelase) were given every 4 hours around the clock. Slight improvement was noted following administration of this drug and muscarinic symptoms were minimal, consisting only of slightly increased salivation. On September 8, 1955, she complained of pain around a tooth and was found to have an interdental periodontal abscess. Intramuscular penicillin administration was started at this time. On September 9, 1955, the abscess was incised and drained. Because of the severity of the myasthenic manifestations, the dosage of WIN 8077 (Mytelase) was rapidly increased. On September 20, 1955, she was taking 75 mg. every 3 hours around the clock and 1 mg. of Prostigmin subcutaneously before each meal. She was discharged on September 23, 1955.

Subsequent course. Following discharge from Sinai Hospital she was advised to take ephedrine sulfate, ¾ grain, three times daily, as well as supplementary potassium chloride in addition to her other medication. Since November, 1955, she was frequently seen and treated at another institution. She had severe and frequent weakness, dysphagia and respiratory distress which were not adequately alleviated by the large amount of administered anticholinesterase medication. Roentgen-ray therapy over the anterior mediastinum was administered in November, 1955, and again subsequently in June, 1956. On each occasion 600 r was administered in divided dosage over a 3-day period. The disease continued to be marked by frequent exacerbations and was particularly aggravated by respiratory infections. In January, 1957, a third course of Roentgen-ray therapy was again administered in the same manner as previously. In July, 1957, spontaneous ecchymoses appeared and she began to notice more marked weakness, as well as marked pallor. Following hematological studies, a diagnosis of acute monocytic leukemia was established. Death from cerebral hemorrhage ensued. At necropsy, in addition to the leukemic manifestations, the microscopic findings in skeletal muscle were reported as follows: "There are definite changes in muscle, focal in nature, consisting of degeneration, loss of striation, increased eosinophils, and proliferation of sarcolemmal cells. Recent necrosis of myofibrils is minimal. No significant infiltration of lymphocytes. Changes are consistent with myasthenia gravis."

Discussion of Cases. This small group of cases displays a wide variation in age of occurrence of the disease, as well as in degree of severity. In Case 3 the disease apparently began in infancy, and in Case 4 it first manifested itself at the age of 64. Enlargement of the thymus was not demonstrated in any of these patients by means of standard chest roentgenograms. Roentgenoscopy with orally administered barium for evidence of thymic enlargement and for evidence of vallecular and pyriform sinus barium retention was carried out in 3 cases (Cases 6, 3, and 1) and revealed barium retention without evidence of thymic enlargement in 2 of these (Cases 6 and 3).

Therapy with Prostigmin alone was adequate in one case (Case 3). Case 6 represents a severe fulminating type of myasthenia gravis, not associated with thymoma, and responding inadequately to treatment with anticholinesterase medication, and perhaps with slight transient remission following Roentgen-ray therapy over the anterior mediastinum. Although death in this patient occurred from another disease, there had been progressive increase in the severity of the myasthenic disorder. Case 3 represents an example of juvenile myasthenia gravis. On the basis of the history, the disease may well have first manifested itself in infancy. The mother was nonmyasthenic. This patient has responded very well to treatment with Mestinon. This favorable response is in accord with the observations of Teng and Osserman[32], who have stated that in their experience the juvenile type of this disease responds well to treatment with Mestinon

and has a better prognosis than the disease in the adult.

Basal metabolic determinations were made in all cases but one (Case 4). In 3 of these (Cases 1, 3 and 6) the determinations were within normal limits. In Case 5, thyrotoxicosis, which began after the diagnosis of myasthenia gravis had been established, was treated first with Tapazole and subsequently with I-131. It is of interest that exacerbations of myasthenic symptoms occurred following treatment of thyrotoxicosis. Of interest also in this case is the fact that there was an exacerbation of myasthenic symptoms on a subsequent hospital admission during treatment of homologous serum jaundice with cortisone.

The subject of the relationship of thyroid activity to myasthenia gravis has evoked considerable interest and investigation. Although muscular weakness has been observed in patients with thyrotoxicosis, this weakness is not of the myasthenic type and is not generally improved by administration of anticholinesterase medication. The coincident occurrence of myasthenia gravis and thyrotoxicosis, though not common, has been observed and reported[25,26,30]. Millikan and Haines[30] have estimated that the incidence of hyperthyroidism before, during and after detectable myasthenia gravis is approximately 5%. Some observers[25,26] have commented upon the frequent "see-saw" relationship between the two diseases. It has been noted that in some cases when myasthenia gravis has preceded the thyrotoxicosis, the onset of the latter resulted in a lessening of the severity of the former. Treatment of thyrotoxicosis in such cases has in some instances resulted in worsening of the myasthenia gravis. However, this relationship is not a constant one. Feinberg *et al.*[13] have reported upon the relationship of myasthenia gravis and myxedema, observing that this combination in their opinion is exceedingly rare. They reported 3 such cases. In 2 of these the myxedema was spontaneous and in the third it was iatrogenic. They do not agree with the concept of a "see-saw" relationship between hyperthyroidism and myasthenia gravis and suggest that treatment be directed at correcting the abnormalities of each disease. They suggest that the relationship between disorders of the thyroid gland and myasthenia gravis are more than coincidental.

Diagnosis. Frequently the diagnosis of myasthenia gravis can be made quite readily on the basis of the clinical features of the disease and prompt improvement upon administration of anticholinesterase medication. Such a situation is exemplified in Case 6. In contrast, a very mild or equivocal case may present considerable diagnostic difficulty. This is exemplified in Case 1.

Case 4 was admitted primarily because of an incarcerated inguinal hernia and, therefore, no problem of diagnosis existed. However, this case does emphasize the importance of prior knowledge of the existence of myasthenia gravis in patients for whom surgery is contemplated. In such situations, additional parenteral treatment is frequently necessary during surgery, especially if the use of muscle relaxants is anticipated. Indeed, the possibility of myasthenia gravis should be thought of in any surgical patient who develops profound muscular relaxation or respiratory difficulty upon administration of small quantities of muscle relaxants. In essence, such a response may be considered a diagnostic test.

1. PROSTIGMIN TEST AND PROVOCATIVE TEST WITH MUSCLE DEPRESSANTS. In 1935, Viets and Schwab[62] introduced the Prostigmin test for myasthenia gravis and this test has remained a

most useful, safe and simple diagnostic procedure that can be carried out readily at the doctor's office or in the patient's home. The technique of this test is well known. In patients with minimal manifestations of the disease, however, this test may not show demonstrable muscle improvement and in such cases provocative tests with quinine or curare may be diagnostic. Provocative tests may be dangerous, however, and should not be undertaken without appropriate precautions and arrangements for prompt treatment in the event of severe respiratory difficulty.

The significance of response to chemical agents is illustrated in all of the cases reviewed. In each of them there was improvement following administration of a stimulating drug. In Case 1, because of a paucity of symptoms or signs upon cessation of treatment with Prostigmin, a depressant drug (quinine) was administered for several days without significant clinical worsening, raising some doubt about the actual presence of the disease, or perhaps indicating transient spontaneous remission in a very mild case of myasthenia gravis.

It has been pointed out in recent publications that some patients with typical manifestations of myasthenia gravis may not respond to Prostigmin. The cause for this failure is not apparent, and the suggestion has been made that such patients may have had polymyositis or muscular dystrophy. Osserman[31] has indicated that this apparent paradoxical situation may be explained on the basis of the fact that in many cases of myasthenia gravis, necrotic and inflammatory muscle changes resembling those seen in muscular dystrophy and in myositis have been demonstrated. Furthermore, responsiveness to anticholinesterase drugs may vary greatly in individual cases. He concludes, therefore, that any syndrome of muscle weakness unaccompanied by alteration of tendon reflexes, in which strength improves upon administration of adequate amounts of Prostigmin or Tensilon, should be considered to be myasthenia gravis. In cases showing typical manifestations of myasthenia gravis with no response to adequate anticholinesterase treatment, classification remains obscure. Rowland[39] has stated that such exceptions do not detract from the general usefulness of the Prostigmin test. It has been stated that in some cases of myasthenia gravis initial unresponsiveness is reversed upon subsequent retesting.

2. TENSILON TEST. In 1951, Westerberg *et al.*[68] investigated the effectiveness of 3-hydroxyphenyltrimethylammonium chloride (Tensilon) in 9 patients with myasthenia gravis. This drug was shown to have the property of effecting prompt but brief, transient improvement upon intravenous administration. Side effects were minimal and the drug appeared to exert its beneficial effect regardless of premedication with other anticholinesterase medication. However, because of rapid dissipation of therapeutic effect, it appeared that this drug had little to offer in the practical long-term management of this disease.

Utilizing these characteristic properties of Tensilon, Osserman and Kaplan[33] devised a test for the diagnosis of myasthenia gravis based upon response to an intravenously administered dose of this drug. The technique was later revised and, in a subsequent report[35], it was indicated that, on the basis of response to fractionally administered doses of Tensilon intravenously, the diagnosis of myasthenia gravis could be established or excluded with rare exceptions. It has been demonstrated that the myasthenic sub-

ject so tested shows prompt temporary increase in muscle strength and subjective improvement without muscle fasciculation. The normal subject so tested usually shows fasciculation without increase in muscle strength. In the psychoneurotic with abnormal fatigability, favorable effects with intravenously administered Tensilon can usually be duplicated by a placebo injection.

The usefulness of this test is further attested by the fact that at times, without such a test, there might be difficulty in the differential diagnosis between myasthenia gravis, psychoneurosis and such diseases as bulbar paralysis, isolated cranial nerve palsies and multiple sclerosis. Such diagnostic problems might at times present themselves by virtue of the fact that any striated muscle in the body may be affected in myasthenia gravis and the disease may thus resemble other neurological entities. The Tensilon test is also useful in differentiating the respiratory crisis which may result either from overdosage with anticholinesterase drugs (cholinergic crisis) or from undertreatment in severe fulminating myasthenia gravis. This is especially pertinent when there are unobtainable or vague histories with reference to previous medication. In such instances, if the crisis is associated with undertreatment, there is prompt transient improvement following intravenous Tensilon and, on the basis of this response, adequate intensive treatment with longer acting medication may then be instituted. If the crisis is due to excessive cholinergic treatment, intravenous Tensilon results in a brief but transient worsening. It is of interest that this principle of utilizing a rapidly acting and quickly dissipated analagous drug to differentiate between undertreatment and overtreatment has been effectively applied recently in problems relating to evaluation of cardiac arrhythmias in heart failure in digitalized patients[24]. Such tests have great merit when properly used, and information thus obtained may at times be lifesaving.

3. DECAMETHONIUM TEST. Response to intravenously administered Decamethonium may serve as a test for myasthenia gravis and for differentiating it from other diseases[8]. This test is based upon the fact that this drug, which produces depolarizing neuromuscular block in the normal subject, produces a somewhat different and variable response in patients with myasthenia gravis. Two main types of response have been described. First, there are those muscles which are extremely resistant to the depolarizing action of Decamethonium and can tolerate many times the amount of the drug required to cause paralysis in the normal subject. At the other extreme, rapid paralysis may occur upon administration of Decamethonium to a myasthenic subject. However, in contrast to the normal subject this weakness can be rapidly reversed by administration of anticholinesterase medication: this in the normal subject results in aggravation of the weakness. It has been postulated that in the subject with myasthenia gravis this drug produces first a brief, fleeting depolarizing block which subsequently changes to a nondepolarizing block, the so-called "dual response"[9].

More recently, Churchill-Davidson[7] has reported a diagnostic response to this test in 42 cases of myasthenia gravis. He considers this test superior to the Prostigmin test as a diagnostic procedure because some cases of myasthenia gravis may fail to show a convincing recovery or improvement upon administration of Prostigmin. He considers the Decamethonium test to be of particular value in distinguishing

early myasthenic weakness from hysterical weakness. Perhaps more significant is the fact that the test has been useful in investigating other diseases with muscle weakness which at times show improvement upon treatment with anticholinesterase drugs. Thus he has reported negative tests in 7 cases of dermatomyositis and 2 cases of thyrotoxic myopathy.

4. ELECTRICAL AND MECHANICAL ACTIVITY OF MUSCLES. Viets[57], in a historical review of myasthenia gravis, points out that Jolly was the first to analyze muscle reaction in this disease. Jolly demonstrated the characteristic rapid fatigue of affected muscles upon continuous or repetitive faradic stimulation.

Following the introduction of Physostigmin and Prostigmin in the treatment of myasthenia gravis, it became possible to demonstrate improvement in muscle strength after administration of these drugs, and Lindsley[23] demonstrated that tension, developed by single twitches in myasthenia gravis patients, could be increased following administration of Prostigmin. In 1952, Botelho *et al.*[5] took electromyograms of the orbicularis oculi muscle in patients with myasthenia gravis and demonstrated progressive decrease in amplitude of successive action potentials following repetitive stimulation of the motor nerves. As Viets[58] has stated, these observations are significant and reliable, but such testing requires elaborate techniques and does not lend itself to routine clinical use. More recently, Botelho[4] has described a technique permitting utilization of simultaneously recorded electrical and mechanical activity. On the basis of tests upon patients with myasthenia gravis and upon partially curarized normal subjects, she concluded that with slow rates of stimulation, tension changes are more accurate indices of muscle weakness than changes in action potential. She demonstrated further that administration of Prostigmin to myasthenia gravis patients resulted in increase in muscle strength as demonstrated by increase in muscle contractability without accompanying increase in action potential. She interpreted this to indicate that weakness in myasthenia gravis may in part be due to changes in contractile ability, apart from changes in neuromuscular transmission.

Eaton and Lambert[12], in a recent study, have shown that electromyographic patterns of a type seen in myasthenia gravis; namely, progressive decline and variation in the amplitude of successive potentials, may be observed in amyotrophic lateral sclerosis, poliomyelitis and in other conditions, and that this defect in transmission may in such cases be reversed by Tensilon or Prostigmin and be accentuated by curare. They have, therefore, stated that, in their opinion, positive results in tests for myasthenia gravis with Prostigmin, Tensilon, and curare are not in themselves pathognomonic of myasthenia gravis. They have described 6 cases in which muscle weakness and electromyographic tests suggestive of myasthenia gravis were associated with intrathoracic malignant tumors of nonthymic origin. However, although these cases were characterized by electromyographic tests similar to those seen in myasthenia gravis, muscle weakness and sensitivity to curare, it is noteworthy that in these cases response to Prostigmin was poor. It may be inferred, therefore, that such a combination of clinical and laboratory features should alert one to the possibility of the presence of intrathoracic neoplasm of nonthymic origin in evaluation of suspected myasthenia gravis.

There have been other recent references to muscle weakness associated

with various types of carcinoma, including bronchogenic carcinoma, showing varying degrees of response to Prostigmin[1,3,17,18,50].

Pathophysiology. 1. NEUROMUSCULAR TRANSMISSION. It is generally believed that the defect in myasthenia gravis consists of an alteration of neuromuscular transmission. In the normal subject, skeletal muscle contraction is associated with the following sequence of events: Upon stimulation of a motor nerve there is release of acetylcholine at the nerve endings. The muscle end-plates are then depolarized by the action of released acetylcholine. End-plate electrical potential is then initiated, causing muscle fiber contraction. As a result of the action of the enzyme acetylcholinesterase, which is present at the motor end-plates, acetylcholine is broken down into acetate and choline. Repolarization of muscle fibers follows and the muscle fibers thus again become susceptible to depolarization. As a result of the action of the enzyme cholinacetylase, acetylcholine is resynthesized from choline and acetate and can then be released in response to a subsequent nerve stimulus. It is to be noted that polarization and repolarization of muscle cells is intimately associated with electrolyte shifts and that electrolyte disorders may greatly influence muscular activity.

Two types of neuromuscular block can be produced in man: depolarization blocks and nondepolarization blocks. The former may be produced by such drugs as acetylcholine, decamethonium and succinylcholine, and the latter by curare and related compounds. On the basis of experimental use of compounds in these two categories in normals and in subjects with myasthenia gravis, some light has been shed on the nature of the defect in myasthenia gravis.

As a result of the studies of Grob, Johns and Harvey[16] some of the differences in neuromuscular transmission between the normal subject and the patient with myasthenia gravis have been elucidated. These investigators studied the response of stimulated muscle in both groups of subjects following administration of acetylcholine, curare, decamethonium and choline. They observed that in normal subjects, as well as in patients with myasthenia gravis, intra-arterial injection of acetylcholine, followed by electrical nerve stimulation, resulted in a brief transient stimulation of muscle activity, followed by a transient depression of activity which could be attributed to the depolarizing action of acetylcholine. This was followed by a temporary recovery of muscle potential, and in turn by a more prolonged "late" depression of induced potentials lasting 30 to 60 minutes. Since the late depression did not begin until several minutes after intra-arterial injection of acetylcholine, and since this depression lasted for as long as one hour (long after hydrolysis of acetylcholine had occurred), it was postulated that the late depression might be caused by the action of a product of the hydrolysis of acetylcholine. Accordingly, sodium acetate and choline chloride were subsequently separately injected and their effects were observed. Although sodium acetate thus injected had no effect, choline chloride produced prolonged depression of induced potentials which resembled the late depression produced by acetylcholine in both normal subjects and in patients with myasthenia gravis. However, while Prostigmin accentuated the acetylcholine-induced prompt depression of muscular activity in both the normal subject and the myasthenic (indicating that a depolarizing block existed), it reversed the late depression in the myasthenic only. In myasthenia gravis patients

choline-induced potential reductions were reversed by Prostigmin or acetylcholine, whereas in the normal subjects they were potentiated by the same procedure. Thus choline, when administered in the above manner, has been shown to reproduce the myasthenic disorder in patients with myasthenia gravis, but not in normal subjects. This led to the assumption that the mechanism responsible for the defect in myasthenia gravis might be that of a competitive ("acetylcholine inhibitory") block, produced by choline released in a normal manner during neuromuscular transmission.

The observations of Churchill-Davidson and Richardson, referred to above, demonstrated the difference between the response of normal subjects and myasthenia gravis patients to the blocking agent decamethonium. In their studies utilizing electromyographic and volitional measurement of muscular activity following intravenous administration of decamethonium in both groups of subjects, they concluded that this drug behaves like a depolarizing agent in normal subjects and behaves ultimately like a competitive blocking agent in patients with myasthenia gravis. They noted that the "dual response" observed by them upon administration of decamethonium to myasthenia gravis patients was analagous to the "prompt" and the "late" depression observed by Grob *et al.*[16] following intra-arterial injection of acetylcholine. Their observations, therefore, also led them to the inference that the pathophysiological basis of myasthenia gravis is an alteration in the response of the motor end-plate to acetylcholine or to a product of its hydrolysis.

2. CIRCULATING CURARE-LIKE SUBSTANCE. The concept of a circulating curare-like substance, possibly of thymic origin, as a cause of myasthenia gravis was postulated following Walker's[64] astute observation that this disease resembled curare poisoning and the observed frequency of thymic abnormalities in this disease. This concept resulted in an effective therapeutic approach to this disease, but thus far has not been substantiated as a cause of it. Churchill-Davidson[7] has pointed out that thus far no extracts from the serum or thymic tissue of myasthenic patients have been found that can reproduce all of the observed characteristics of curare-induced neuromuscular block; namely, (1) prompt reversibility of the block upon administration of Prostigmin or Tensilon; (2) the greater ability of slow stimuli to traverse the block as opposed to rapid stimuli; (3) the constant capability of muscle contraction upon direct stimulation, even though there may be failure to respond to nerve stimulation.

Wilson and Wilson[70] have had the greatest experience with extracts from thymus glands removed from patients with myasthenia gravis, and have shown that these extracts can produce neuromuscular block in animal preparations. Such block, however, differs from that seen in myasthenia gravis and in curarized animal preparations in that it is not reversed by Prostigmin. Wilson[69] has also noted that thymus gland extracts from nonmyasthenic adults, obtained at necropsy, show little or no neuromuscular blocking action, whereas the degree of neuromuscular blocking activity of extracts from glands removed from patients with myasthenia gravis is correlated with the degree of postoperative improvement in such patients. It would appear, therefore, that a substance capable of causing neuromuscular block can be extracted from the thymus glands of patients with myasthenia gravis, but that the block thus produced does not resemble curare-

induced block. A similar substance has been extracted from fetal whale thymus. Extracts of other human tissue, such as lymph nodes and muscle, have not been demonstrated to possess such property.

Zack and Cohen[71], testing acetone extracts prepared from the thymus glands of patients with myasthenia gravis, as well as the thymus gland of one normal child, and striated muscle and normal spleens, noted that both acetone-soluble and acetone-insoluble fractions of these tissues decreased the muscle tension of an isolated frog sartorius muscle within 20 to 30 minutes. When the muscle became inexcitable on indirect stimulation, it remained inexcitable on direct stimulation. The active material in the extracts was heat-stable, dialysable and was antagonized by the addition of calcium chloride to the extracts. It was noted that these extracts contained from two to twenty-three times the potassium concentration of frog Ringer's solution, and that these concentrations of potassium were sufficient in themselves to produce the observed muscle inhibition. When equivalent amounts of potassium chloride were added to frog Ringer's solution, inhibiting effects comparable to those observed with thymic extracts were obtained. They concluded that the presence of large amounts of potassium in the thymus gland extracts from myasthenia gravis patients does not permit an evaluation of other possible fractions in such extracts which might have muscle contraction inhibitory properties.

Pathology. Until fairly recently, the only pathological changes described in the muscles of patients with myasthenia gravis have been lymphorrhages. In addition, the relatively frequent presence of thymic disorders in this disease has long been known[65]. Querido[38], as early as 1929, in a necropsied case of myasthenia gravis, demonstrated degeneration of myocardial and muscular tissue as well as perivascular cellular infiltration with fibrous tissue proliferation. He noted similar changes in other organs and suggested that myasthenia gravis might be considered as a generalized vascular disease having the pathological characteristic of chronic proliferating perivasculitis.

More recently, others[27,43,51] have demonstrated changes in the myocardial and muscle tissue of patients with myasthenia gravis. Russell[43] has classified histological muscle changes in myasthenia gravis as follows:

Type I. Cases showing fibrinous necrosis and inflammatory cellular reaction.

Type II. Cases showing progressive atrophy of muscle fibers with late formation of lymphorrhages.

Type III. Cases showing simple atrophy affecting single fibers or groups of fibers.

Mendelow and Genkins[27], describing the pathological changes in 12 consecutive necropsies of myasthenia gravis patients, reported necrotic changes in the muscles and myocardium in 6 cases. Myocardial changes were noted in each of 4 cases with thymoma. Rowland and Eskenazi[40] have described a patient demonstrating some features resembling muscular dystrophy and others suggestive of myasthenia gravis. The patient responded strikingly to Tensilon and Prostigmin and showed marked sensitivity to curare. Muscle biopsy showed foci of muscle degeneration and sarcolemmal nuclear proliferation, which were interpreted as being compatible with the diagnosis of muscular dystrophy. The patient had marked weakness of the shoulder and pelvic girdles

with waddling gait, lordosis and inability to arise from supine or squatting positions. In addition, there was marked creatinuria, all factors compatible with muscular dystrophy. Features favoring myasthenia gravis were minimal muscular wasting, presence of long-standing ophthalmoplegia, preservation of tendon reflexes, and favorable response to anticholinesterase drugs.

Rowland *et al.*[41], reviewing the findings at necropsy in 26 cases of myasthenia gravis, reported skeletal muscle lymphorrhages in 16 cases. Some of these were perivascular in distribution. Muscle fiber abnormalities, consisting of shrinking, swelling and loss of striation, were noted in 12 cases. Definite muscle necrosis was present in 3 cases and was associated with leukocytic infiltration about the degenerating fibers.

Antemortem tissue biopsies were not obtained in any of the presently reviewed cases. However, at necropsy Case 6 did show, in addition to the skeletal muscle changes described in the case summary, persistent islands of thymic tissue in adipose tissue consisting of epithelial and lymphocytic elements, with preservation of Hassell's corpuscles. Although the heart showed cloudy swelling and some degenerative lipidic infiltration of myofibrils, these changes were not considered to be attributable to myasthenia gravis.

It must be observed that pathological changes of the type described above do not occur universally in this disease, and in themselves cannot be considered as being pathognomonic of myasthenia gravis. Perhaps, as suggested by Paterson[37], more frequent histological study of biopsied muscle tissue in live patients with myasthenia gravis, utilizing newly developed techniques, might help in elucidating the pathology and pathophysiology of this disease. He favors the concept that myasthenia gravis is not a disease of specific etiology but a syndrome of varied etiology.

The Role of the Thymus Gland in Myasthenia Gravis. Osserman[31] has recently stated that "there is no more confusing problem in myasthenia gravis than that of the thymus gland, and its relationship to myasthenia gravis."

Viets[59], emphasizing the large incidence of thymic disorder in this disease, as well as the large incidence of malignant thymomas in patients with thymic enlargement, asserts that there must obviously be a significant relationship between the thymus gland and the disease myasthenia gravis. Just what this relationship may be is not apparent, although it has been observed and reported that in myasthenia gravis patients with thymomas, the disease is generally more severe[21].

It has been estimated[19] that 50 to 75% of myasthenia gravis patients have enlargement of the thymus and that 15% of this group have thymomas. It has been further estimated[6] that in about 25% of the cases with thymomas there may be extension beyond the confines of the gland to surrounding structures. Reference has been made above to studies conducted with extracts of thymus glands from normal subjects as well as from patients with myasthenia gravis.

As to the matter of benefits resulting from the surgical removal of thymus glands in patients with myasthenia gravis, considerable difference of opinion exists. The first successful removal of a tumor of the thymus gland in a patient with myasthenia gravis was reported in 1939[2]. Subsequently there have been many reports of results of thymectomy in patients with myasthenia gravis. These have included operations done for patients with and without thymomas.

In an analysis of surgically treated

cases as compared with nonsurgically treated cases, considering age, sex, age of onset of disease and duration of disease, Schwab and Leland[46] concluded as follows:

1. Patients with thymoma were generally not benefited by thymectomy.
2. Best results from thymectomy occurred in female patients under 30 years of age, who did not have thymomas and in whom the duration of the disease was less than 5 years.
3. In male patients over 30 years of age, thymectomy did not favorably alter the disease.

Eaton and Claggett[11], in a review of the present status of thymectomy in myasthenia gravis, analyzed their series of surgically treated cases and a medically treated control group, and compared their results with those of others[10,14,22,46,49]. This study reveals that evaluation of "results" is frequently a difficult matter, inasmuch as each investigator may have a somewhat different criterion as to what constitutes a "good" or "bad" result. They indicate that, although thymic disorder is not necessarily the cause of myasthenia gravis, they are attracted to the theory that the basic disorder in endocrine metabolism which may be responsible for myasthenia gravis may also give rise to changes in the thymus gland. Removal of the thymus gland, therefore, when effective, may in their opinion reverse the endocrine metabolic disorder with resultant lessening of the severity of myasthenia gravis. They conclude, from the evaluation of their data, as follows:

1. Thymectomy is of value in the treatment of female patients with myasthenia gravis who are less than 50 years of age and who do not have thymomas. Thymectomy is not indicated in such patients if the disease has been present for 10 years or more, and if the condition is stationary or improving.
2. Although benefit from thymectomy in male patients has not been generally noted, this operation is offered to male patients unless the surgical risk is too great.
3. Thymectomy for removal of thymic tumors, although it carries a relatively great risk to the patient, may be considered, provided there is no roentgenological or clinical evidence of inoperability, and provided that the risk of surgical intervention is not excessive. The rationale for this attitude is the premise that, if unoperated, such patients may die of their thymomas, whereas if they should survive the operation, they may do well as far as the myasthenia gravis is concerned. For patients with inoperable thymomas, Roentgen therapy is given.

The question as to why only females, and not males, may be benefited by thymectomy, and as to the variability of results from this operative procedure, both in patients with and without thymoma, remains unanswered.

Treatment. 1. PROSTIGMIN. The era of effective treatment of myasthenia gravis began in 1934 following Walker's[64] observation that the weakness in this disease resembled curare poisoning, and could be alleviated by the administration of the tertiary ammonium base, physostigmin. In the following year[63] she reported more favorable results following treatment with a newly available synthetic drug, the quaternary ammonium base, neostigmin. Neostigmin has remained a most effective drug in the treatment of this disease and may be administered orally or parenterally. The dosage depends upon the severity of the disease and, in general, the drug is very effective. Overdosage may result in so-called "side effects" which are really manifestations of potentiation of endogenous acetylcholine effects resulting

from inhibition of acetylcholine hydrolysis. These effects have been called "muscarinic" effects and "nicotinic" effects. In addition, central nervous system effects have also been noted. "Muscarinic" or muscarine-like effects, consisting of nausea, diarrhea, cramps and vomiting, result from smooth muscle stimulation of the gastrointestinal tract. Similar stimulatory effects on the bronchial musculature may produce bronchoconstriction and the effects upon the salivary and bronchial secretory glands may result in excessive salivation and bronchial secretion. Myosis results from the effects upon the pupils. Central nervous system effects are variable and may consist of restlessness, giddiness, headache, confusion, stupor, coma and convulsions. "Nicotinic" effects consist of muscular weakness and fasciculation and slurred speech and dysphagia. Some of the disadvantages of Prostigmin in the treatment of myasthenia gravis are its relatively short period of action, and of resistance to the effects of the drug in severe cases of myasthenia gravis. In addition, there is a narrow margin between full therapeutic and toxic effects. The relatively brief period of activity of the drug at times necessitates frequent administration around the clock in severe cases of myasthenia gravis.

These disadvantages of Prostigmin in severe myasthenia gravis are exemplified in Cases 5 and 6. By contrast, effectiveness of this drug in a case of moderate severity in an adult patient with myasthenia gravis is illustrated in Case 2.

2. MESTINON (PYRIDOSTIGMIN BROMIDE). This drug is also a quaternary ammonium base (dimethylcarbamic ester of 3-hydroxy-1-methylpyridinium bromide) which was synthesized in 1945 and has subsequently been found to be very effective in the treatment of myasthenia gravis. Reports by Osserman[36], Schwab and Timberlake[48], Tether[53] and Westerberg *et al.*[67] reflect the wide interest in this drug. Osserman[32] has found that although the duration of action of Mestinon during daytime administration only slightly exceeds that of Prostigmin, its nocturnal effect is greater, at times permitting a full night's sleep without severe morning weakness. The reason for this effect is not apparent. He has also noted that this drug is more effective than Prostigmin in the relief of the weakness of muscles innervated by the cranial nerves. Another observed advantage of Mestinon over Prostigmin is the wider range between therapeutic and toxic effects. However, toxic effects can and do occur with Mestinon as with any effective anticholinesterase medication. Although he believes Mestinon to be superior to Prostigmin in the treatment of myasthenia gravis, he has stated that it is unlikely that Mestinon or any other anticholinesterase drug has been found to be effective in patients who are resistant to Prostigmin. Teng and Osserman[52] consider Mestinon the drug of choice in juvenile myasthenia gravis.

3. MYTELASE. (Formerly known as WIN 8077 or Mysuran chloride). In 1953, another effective synthetic anticholinesterase compound was made available for the treatment of myasthenia gravis. This drug is now called Mytelase. Chemically, it is N, N^1-bis (2-diethylaminoethyl) oxamide bis-2-chlorobenzylchloride. It has been found to have a much greater anticurare effect than either Tensilon or Prostigmin and its anticholinesterase activity is approximately equivalent to that of Prostigmin. Its duration of action is considerably longer than that

of Prostigmin, but only slightly greater than that of Mestinon. Toxic effects occur as they do with any other anticholinesterase medication. However, excessive salivation is apt to occur before abdominal cramps or diarrhea. Furthermore, muscle fasciculation and bradycardia may also occur before abdominal cramps and diarrhea. For these reasons, as well as because of its longer duration of action, this drug should be administered with caution and under close observation until required dosage and patient response are clearly established.

Schwab[45] has reported that after transfer from other medication, 59 out of a group of 75 patients preferred this drug, although 17 of this group were taking it in combination with Mestinon or Prostigmin.

Westerberg[66] reported that in a group of 33 patients to whom she had given this drug, 7 found it to be ineffective and 26 found it to be effective. Twenty-two of her patients found this drug to be either equal to or more effective than other anticholinesterase medication.

Cases 5 and 6 in our group noted better relief of their symptoms after transfer to Mytelase, and required less frequent administration of medication than was the case when they were taking Prostigmin. However, in both patients frequent supplementary administration of Prostigmin, orally and parenterally, was necessary because of its prompt effectiveness, especially during relapse. In Case 4, treatment was started and maintained effectively with Mytelase alone.

4. NEW DOSAGE FORMS OF PROSTIGMIN AND MESTINON. Recently Schwab, Osserman and Tether[47] have reported on the effectiveness of treatment of myasthenia gravis with specially prepared multiple dose tablets designed in an effort to lessen the frequency of administration of anticholinesterase medication, particularly nocturnal administration. They administered slow release tablets of Prostigmin, containing three regular doses of 15 mg. each, as well as half-strength slow release tablets containing three doses of 7.5 mg. each. Similar tablets of Mestinon were also tested. These forms of medication are most effective in decreasing the frequency of nocturnal treatment, permitting longer intervals of uninterrupted sleep. The special tablets of Prostigmin were tried in 85 patients, 54 of whom expressed preference for this form at the conclusion of the trial period. Eighty-two of 109 patients who were tested with long-acting Mestinon tablets found them beneficial and continued to take them after the conclusion of the trial period. The average duration of effectiveness of these slow release tablets was found to be approximately 6 hours.

5. NEW COMPOUNDS. Osserman[31] refers to experimental work with new compounds produced by alterations of the Prostigmin molecule. Many modifications have been tried in attempts to find a therapeutic agent that will have a longer duration of effect and less toxicity than currently available drugs. There are to date insufficient data for evaluation of these compounds.

6. ORGANIC PHOSPHORIC ACID ESTERS. Drugs of this category have been used in the treatment of myasthenia gravis. They differ from the quaternary ammonium compounds structurally as well as pharmacologically. The quaternary ammonium compounds exert their therapeutic effect by virtue of a reversible combination with cholinesterase enzymes, whereas organic phosphoric acid esters form a more lasting

combination with these enzymes, which is frequently irreversible. Furthermore, organic phosphoric acid esters are generally highly soluble in lipoid media and therefore have more marked central nervous system effects than the quaternary ammonium compounds which are highly soluble in aqueous media and, in general, have much less effect on the central nervous system. Therefore, the usefulness of these compounds, is greatly limited by their toxicity. Drugs of this group which have been tried in the treatment of myasthenia gravis are tetraethyl pyrophosphate (TEPP), hexaethyltetraphosphate (HETP), diisopropyl fluorophosphate (DEP) and octamethyl pyrophosphoramide (OMPA). Schulman *et al.*[44] stated that 7 deaths in a group of 15 patients treated with OMPA were due to intoxication with this drug. Osserman and Kaplan[34], summarizing their experience with this drug, found also that it is too toxic to warrant its continued use. They transferred some of their more seriously ill patients, who were nonresponsive to Prostigmin, to OMPA. Of 12 patients so treated, 5 died. Two deaths were due to cholinergic crisis, attributable to OMPA intoxication, and 2 deaths were attributed to malignant thymomas with metastases. The fifth death occurred in an uncontrollable myasthenic who developed terminal bronchopneumonia. These authors emphasized the fact that the toxic effects of OMPA are muscarinic reactions, consisting of salivation, nausea, vomiting, increased peristalsis, diarrhea and diaphoresis, and that even with the addition of atropine sulfate, these toxic effects were not sufficiently suppressed to warrant the continued use of this drug. The manufacture of compounds of this group is attended with considerable hazard to the personnel so employed, and for this reason some of these compounds have been withdrawn.

7. CORTISONE AND ACTH. In a review of the effect of these hormones on myasthenia gravis, Merritt[28] noted that in most instances, following treatment, there was temporary exacerbation followed by brief incomplete remissions lasting several weeks to several months. Grob and Harvey[15] noted no improvement either during or after use of these hormones, and in fact observed exacerbations of the disease in a number of patients so treated. Torda and Wolf[56] administered ACTH to patients with myasthenia gravis on the basis of the following rationale:

1. Administration of ACTH causes shrinkage of the thymus and enhances synthesis of acetylcholine.

2. Removal of the pituitary gland in rats produces changes in the electromyogram, which resembles those seen in patients with myasthenia gravis.

They administered ACTH to each of 15 patients and obtained significant remission in 10 cases.

Only one patient of the group presently reviewed received cortisone (Case 5). It is noteworthy that, during the period of cortisone administration, requirement for anticholinesterase medication increased and persisted for approximately one week after cortisone was discontinued.

Kane[20], in a discussion of the effect of endocrine glands on myasthenia gravis, tabulated the reported results of treatment of this disease with cortisone and ACTH by many investigators. He concluded that results with cortisone are disappointing, and that with

the use of ACTH there may be initial worsening, slight improvement upon cessation of therapy, and at times striking improvement upon cessation of therapy. On the other hand, several deaths have been reported during ACTH therapy.

Osserman[31] observes that current opinion is against the use of ACTH in myasthenia gravis *per se*. He states that its use may be indicated when a patient with myasthenia gravis develops an inoperable thymic tumor or any secondary disorder which in itself would be benefited by the administration of ACTH.

Based on the premise of possible favorable effect of treatment of myasthenia gravis with ACTH, the operation of carotid sinus denervation has been tried in the treatment of this disease. The rationale for this operation is based upon animal experimentation, indicating that carotid sinus denervation is followed by adrenocortical hypertrophy. Reports of this procedure have been published by Thevenard *et al.*[55] and by Mertens[29]. The operation is recommended as being indicated for patients who do not respond well to medical treatment and who have frequent relapses. Referring to this operation, Viets[60] has stated that its results are minimal and that until more information is available, the operation cannot be recommended.

8. ROENTGEN-RAY THERAPY TO THE MEDIASTINUM. Roentgen-ray therapy has been used in the treatment of myasthenia gravis for many years. It has demonstrated some degree of usefulness in two groups of cases; first, in the treatment of thymomas. In such cases, the dosage is quite high, generally 3000 to 4000 r over a period of 4 to 6 weeks. Remissions have been noted in some cases following such radiation therapy alone. In others, if remission is not observed or sustained, Roentgen-ray therapy has been followed by thymectomy.

Another field of usefulness of Roentgen-ray therapy is in the treatment of myasthenia gravis without thymoma. Roentgen-ray therapy is administered over the region of the thymus to the anterior mediastinum on several separate occasions for a total dosage of approximately 600 r during each treatment period. This form of therapy has been used in all degrees of severity of the disease and at times results in satisfactory remissions. Case 6 demonstrates the temporary effectiveness of this form of therapy.

9. TREATMENT OF MYASTHENIC CRISIS AND CHOLINERGIC CRISIS. Tether[54] recently summarized present-day treatment of myasthenic and cholinergic crises. As a basis for effective treatment, appreciation of basic considerations is important. First, the term "crisis" is used to designate, in the myasthenic patient, a state in which there is sufficient failure of the muscles of respiration or deglutition, or both, to the extent that their function cannot be maintained without mechanical aids. Secondly, it is known that excessive administration of cholinergic medication may result in weakness which clinically may resemble that seen in undertreated or untreated myasthenic crises. In differentiating these two conditions, as well as evaluating the need for more medication, the Tensilon test of Osserman, described above, is a most helpful procedure. Tether has recommended that when crisis occurs, a tracheotomy or a tracheal intubation must be accomplished promptly. This permits an airway through which secretions can be aspirated and oxygen administered. If

voluntary breathing is inadequate, the patient should be placed in a respirator. In the presence of excess secretions, bradycardia and myosis, atropine sulfate may be administered. If no improvement follows, the Tensilon test should be done. If improvement follows this test, the crisis is probably myasthenic and more vigorous anticholinesterase therapy instituted. If, following the Tensilon test, muscle fasciculation occurs and other symptoms are aggravated, the crisis is probably cholinergic. This is generally treated with repeated doses of atropine sulfate. Cholinergic medication is discontinued until improvement occurs, followed by a return of myasthenic weakness, at which time cholinergic medication is cautiously resumed. When the patient is in the respirator, foods, fluids and electrolytes should be administered intravenously and antibiotic therapy administered. When the respirator is not constantly necessary, tube feeding may be started. The occurrence of atelectasis or bronchial obstruction may necessitate bronchoscopy as a life-saving measure. When cholinergic medication is again instituted, the requirements may be gauged by repeated Tensilon tests. If medication cannot be taken orally, it may be given either subcutaneously or with intravenous fluids, as originally recommended by Viets[61].

Patients with myasthenia gravis should be taught to recognize cholinergic crisis (perhaps as diabetics learn to differentiate hypoglycemic and hyperglycemic weakness), and be given atropine or belladonna for prompt administration should such situations arise. However, atropine and belladonna should not be taken regularly, inasmuch as they may mask or obscure the muscarinic symptoms of cholinergic overdosage and permit precipitation of cholinergic crises.

Conclusions. 1. Careful clinical appraisal and response to anticholinesterase remain the basis for diagnosis in the majority of cases of myasthenia gravis. At times, in very mild or equivocal cases, the use of a provocative test, with quinine or curare, may be useful. Such tests should be carried out wih appropriate care and precaution.

2. Recent investigative work has shed some light on the nature of the pathophysiology of this disease, but the etiology remains obscure.

3. New drugs, introduced during recent years, as well as newer methods of administration of medication, particularly multiple-dose, slow-release tablets, have much to offer in the management of the symptoms of myasthenia gravis.

4. The exact relationship of the disease myasthenia gravis to the thymus gland and to the endocrine system remains obscure.

ACKNOWLEDGMENT: I am indebted to Drs. I. Donald Fagin, Harold I. Ginsberg, Abe S. Goldstein, Stanley H. Levy and Myer Teitelbaum of the medical staff of Sinai Hospital, Detroit, for permission to review their patients' charts; and to Drs. Russell N. Dejong, Martha R. Westerberg, and A. James French of the University Hospital, Ann Arbor, Michigan, for permission to utilize personal communications pertaining to Case 6. I also wish to thank Dr. J. Edward Berk, Chief of the Division of Medicine, Sinai Hospital, Detroit, for his advice and assistance.

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BOOK REVIEWS AND NOTICES

SEKRETIONSSTUDIEN AM PANCREAS. By DR. MED. VOLKER BECKER, Pathological Institute, University of Kiel. Pp. 119; 54 ills. Stuttgart: Georg Thieme Verlag, 1957 (Intercontinental Medical Book Corporation, New York, U.S. Agents). Price, $5.60.

THIS monograph, written in German, is an attempt on the part of the author to better correlate structural and functional changes of the pancreas. Towards this end he has performed many studies in animals using a great number of pancreatic stimuli (ethionine, atropine, alloxan, mecholyl, pilocarpine, and others) followed by gross and microscopic examination of the pancreas. These are beautifully illustrated by 54 photomicrographs.

The second portion of this work is devoted to an excellent description of the histological findings in various clinical conditions of the pancreas such as old age, chronic relapsing and acute hemorrhagic pancreatitis. These are obtained from 76 case presentations.

There is a complete historical review of each subject discussed, and the Bibliography is excellent. The fact that this work is in German will unfortunately limit its usefulness. It is highly recommended to those interested in the pancreas from an experimental or clinical point of view. D. B.

SPRACHAUDIOMETRIE. By DOZ. DR. MED. KARL-HEINZ HAHLBROCK. Pp. 182; 123 ills. Stuttgart: Georg Thieme Verlag, 1957 (Intercontinental Medical Book Corporation, New York, U.S. Agents). Price, $5.00.

IN properly selected cases, transtympanic surgery brings about restoration of hearing in the majority of patients with conductive deafness. The techniques of Lempert, Rosen, Zöllner and Wullstein have brought about this achievement. Some cases are readily identified as otosclerosis, while others present considerable difficulty. Speech audiometry, threshold and discrimination score, constitute most important links in the identification of a case of conductive deafness, which may not be denied the benefits of the new operations.

Dr. Hahlbrock's volume describes all the accepted techniques of speech audiometry. The diagnostic implications are well presented. His "word lists" in German are excellent. The illustrations are beautiful and the Bibliography and Index are adequate. M. S.

COLD INJURY. Transactions of the Fourth Conference, November 7, 8 and 9, 1955. Edited by M. IRENÉ FERRER, M.D. Pp. 371; 134 ills. New York: Josiah Macy, Jr. Foundation, 1957. Price, $5.95.

THIS volume, like the preceding three transactions, is a stenographic report of a round table conference on cold injury. The participants are investigators and clinicians actively engaged in the field of hypothermia.

This report, to a greater degree than the three preceding reports, contains information of immediate clinical importance. For example, there are detailed data on the late effects of frostbite, the roentgenologic changes in bones following cold injury in man, the resuscitation of frozen mammals, clinical application of hypothermia during open heart surgery, blockade techniques against ventricular fibrillation during hypothermia, and cerebral studies during local and general hypothermia. In addition, there are several chapters on experimental cold injury in animals.

The informal presentation with free discussion among the members makes the reader feel that he is actually sitting in on the conference. These transactions are invaluable to any physician who is interested in cold injury and in the application of hypothermia to open heart surgery. N. L.

MANAGEMENT OF COMPLICATIONS IN EYE SURGERY. Edited by R. M. FASANELLA, M.D., Chairman, Section of Ophthalmology, Yale University School of Medicine. Pp. 422; 155 ills. Philadelphia and London: W. B. Saunders Co., 1957. Price, $16.00.

COMPLICATIONS in eye surgery are an unwanted biproduct of active practice. There are many books dealing with proper management of routine problems. This enables the surgeon to steer clear of complications in a very high percentage of cases.

Even when the diagnosis is correct and the technique faultless, difficulties will arise. A text of this type is of great value. It outlines the possible complications in each type of procedure; tells you how to avoid them;

and when unwanted problems exist, what to do about them.

This book is equally as necessary as a text outlining surgical procedure. There is no question that a knowledge of possible complications and what to do about them tends to minimize problems and get proper treatment instituted early.

The contributors are expert in their fields and their reviews are informative but not cumbersome.

This is a worth-while book for every ophthalmologist. L. H.

SELECTED WRITINGS OF WALTER E. DANDY. Compiled by CHARLES E. TROLAND, M.D., AND FRANK J. OTENASEK, M.D. Pp. 789; illustrated. Springfield, Ill.: Charles C Thomas, 1957. Price, $15.00.

THIS compilation is dedicated to the memory of Dr. Walter E. Dandy, a pioneer and gifted neurosurgeon. The funds for its publication were donated by his immediate family, some of his residents, and friends.

The contents are divided into 78 chapters and each presents a subject published by Dr. Dandy in some medical or surgical journal. The material is diversified and covers almost every condition encountered in neurosurgery.

Dr. Dandy was an innovator, a master surgeon and he has a great many "firsts."

His greatest single contribution toward diagnosis in neurology and neurosurgery was his development of the procedures of encephalography and ventriculography.

He was the first to treat Meniere's disease by section of the vestibular portion of the acoustic nerve. He developed the posterior fossa approach for section of the 5th nerve in trigeminal neuralgia. He was the originator of the high spinal intracranial operation for spasmodic torticollis and he attacked the problem of intracranial aneurysm before the popularization of cerebral angiography and the advent of the newer methods of anesthesia and hypothermia.

Drs. Troland and Otenasek should be thanked for bringing together Dr. Dandy's important contributions. There is an inexhaustible storehouse of knowledge which will be of great value and interest to the neurosurgeon, neurologist, and neurophysiologist. This volume is indispensable to the resident starting in the field of neurosurgery.

M. S.

THE CLINICAL ASPECTS OF ARTERIOSCLEROSIS. BY SEYMOUR H. RINZLER, M.D., Associate Physician, Beth Israel Hospital, Associate Visiting Physician, Bellevue Hospital. Pp. 339; 57 ills.; 11 color plates. Springfield, Ill.: Charles C Thomas, 1957. Price, $8.75.

SINCE arteriosclerotic disease has achieved undisputedly the title of Captain of the Men of Death in our present civilization, and since it is a leading cause of morbidity as well, not only in our elderly but also in our middle-aged population, a book of this type written for the general practitioner is to be welcomed, and should be very popular. The author has surveyed the massive literature on the subject and has wisely selected the material suited to the clinical understanding of degenerative arterial diseases. The clinical emphasis, however, has not precluded an inclusion of recent basic discoveries along morphological, physiological, biochemical, geopathological and epidemiologic lines, and this is found to represent a very careful culling of information which is likely to stand the test of time. He does not accept the formerly dominant nihilistic view that atherosclerosis is an almost inevitable concomitant of senescence and shows that it is a potentially reversible process.

From the clinical standpoint arteriosclerotic heart disease receives particular emphasis, but other portions of the body have not been neglected. There is presented the diagnostic and therapeutic aspects of the major and minor manifestations of arteriosclerosis literally from head to foot. Excellent illustrations, some in color, and a good Index add materially to the value of the volume. This book is highly recommended for the general practitioner, and the specialist will undoubtedly find much of value, including the excellent Bibliography. T.D.

CORTISONE THERAPY MAINLY APPLIED TO THE RHEUMATIC DISEASES. By J. H. GLYN, M.D., M.R.C.P., Consultant in Physical Medicine to the Prince of Wales and Tottenham Group of Hospitals, London. Pp. 162; 11 ills. New York: Philosophical Library, Inc., 1957. Price, $10.00.

THIS small volume, which is written in a clear and interesting style, brings together an extraordinarily large amount of information regarding the early history, the chemistry and pharmacology of cortisone. Also, it deals incisively, fairly and exhaustively with the problems of cortisone management of the rheumatic diseases. Perhaps the most valuable part of the book, for the rheumatologist at least, is the author's personal experience with

the difficult problems of when and how to administer cortisone.

However, the American reader will miss a full discussion of both the newer, double-bonded corticosteroids and the use of phenylbutazone in rheumatoid spondylitis. Also, he may feel that the author has failed to come to grips with the most important question of all, namely the real results of cortisone therapy of rheumatoid arthritis over periods of 5 or more years. J. L.

THE HUMAN EAR CANAL. By ELDON T. PERRY, M.D., Instructor in Dermatology, Wayne University. Pp. 116; 40 ills. Springfield, Ill.: Charles C Thomas, 1957 (Published simultaneously in Oxford, England: Blackwell Scientific Publications, Ltd. and in Toronto, Canada: The Ryerson Press). Price, $4.75.

PERRY's The Human Ear Canal is a study of this part of the anatomy with information valuable to all physicians. The book contains a detailed discussion of the anatomy, physiology and microbiology of the ear canal. There is an up-to-date compilation of the known on cerumen. The clinical picture of external otitis, its experimental production, diagnosis and treatment are also included. These data are documented by an adequate bibliography at the end of each chapter and additional bibliography at the end of the book. A general index of subject and authors is appended. This is a beautifully illustrated and well-written volume. Except for a lack of specific details in the therapy chapter, it is a comprehensive treatise on the human ear canal.

H. B.

BONE DISEASES IN MEDICAL PRACTICE. By I. SNAPPER, M.D., Beth-El Hospital, Brooklyn, New York. Pp. 277; 48 plates. New York: Grune & Stratton, Inc., 1957. Price, $15.00.

THE book consists of 28 chapters on diseases in which lesions may be expected to occur in the skeleton. Some of these, as cortical hyperostosis and Paget's disease are primarily bone diseases; others such as renal osteitis fibrosa (secondary parathyroid hyperplasia, renal rickets) and Boeck's sarcoidosis are diseases in which the skeleton may or may not be involved. There is a chapter on the physiology of bone, on the semantics of bone disease and one on the differential diagnosis of bone lesions. At the end of the book there are 48 pages of illustrations, almost all of them roentgenograms.

Since in this rather slender volume, some 28 different disease entities are discussed, it is obvious that few of them are given a complete treatment. One gets the impression that the author has written as he pleased and about what he wanted to without much attention to the conventions of authorship. Some of the chapters are no longer than two pages. There are few writers other than Dr. Snapper who could write such a book and produce something of value. This book is a treasure, though a small one, because it records the opinions and experience of one of the world's great clinicians and teachers. Without bothering about the fundamentals he records his observations in a number of diseases and the whole adds up to a valuable collection of signs and symptoms of which the modern clinician must be aware. If the tone of complete assurance disturbs the reader, he must remember that the author is writing from an enormous experience which few people ever hope to attain. E. A.

BONE TUMORS. By DAVID C. DAHLIN, M.D., Mayo Clinic. Pp. 224; 258 ills. Springfield, Ill.: Charles C Thomas, 1957. Price, $11.50.

BONE TUMORS is essentially an atlas, which records the data obtained by an analysis of the Mayo Clinic material of over 2,000 cases. It is a good atlas because it contains enough text to explain the illustrations. A preliminary chapter gives a classification and maps the structure of presentation in the remainder of the book. A chapter is devoted to each entity and each chapter contains a diagram of the age, sex and site incidence, illustrative roentgenograms, gross and sometimes clinical pictures and several photomicrographs. The book is an intelligent survey of one of the largest collections of bone tumors in the world, much of which has been presented in the journals of the past several years, put together in an orderly fashion in a single volume. Because follow-up study on most of the cases was available the author includes a paragraph on treatment and one on prognosis for each entity. E. A.

GENERAL DIAGNOSIS AND THERAPY OF SKIN DISEASES. By HERMANN WERNER SIEMENS, M.D., Professor of Skin and Venereal Diseases, University of Leiden, Holland. Translated from the German by KURT WIENER,

M.D., Dermatologist, Mt. Sinai Hospital, Milwaukee, Wis. Pp. 324; 375 ills. Chicago: University of Chicago Press, 1958. Price, $10.00.

THIS beautifully illustrated and well printed volume is a useful source book for teachers of dermatology who wish detailed information on the basic lesions as an aid in preparation of their lectures on the physical diagnosis of dermatology. However, the reviewer doubts that it, presenting a new approach to the teaching of dermatology by concentrating on the morphologic fundamentals of skin disease, will be of much use as a textbook for students or practicing physicians, since detailed discussion of individual diseases is not included. The idea of emphasizing morphology is laudable, but modern trends in dermatology stress, in addition, the underlying causes and mechanisms producing the fundamental lesions. This book does not cover this phase of the subject. The content is divided into general diagnosis (including a discussion of color; lesions; extent, shape and distribution; hair and nails; systemic symptoms; subjective symptoms and history; and auxiliary diagnostic techniques), and principles of therapy (including vehicles; medications in the stricter sense [active ingredients]; administration of treatment; physical therapy; minor surgery in skin diseases; systemic treatment of skin diseases; and therapy and experience). This text material is introdced by an all too brief discussion of anatomy and histopathology of the skin. The subjects are well indexed. Although this work is a translation from German, the book reads well due to the devotion of a skillful translator who is himself author of successful dermatologic books, Kurt Wiener. H. B.

MODERN TRENDS IN NEUROLOGY. Edited by DENIS WILLIAMS, C.B.E., M.D., D.Sc., Physician, National Hospital, Queen Square. Pp. 350; 80 ills. New York: Paul B. Hoeber, Inc., 1957. Price, $13.50.

THIS valuable volume is another in the series of modern trend in neurology. It is a satisfactory and authoritative review of the urgent problems of neurology. It contains, among other subjects, a survey of clinical electromyography, a subject which presumes to displace clinical observation by mechanical recordings. The section relating to this problem ends with a discussion of its future and quotes Sir E. D. Adrian as follows in his Oliver Sharpey lectures: "The subject of these lectures is not one in which striking advances have been made in the last few years. It is one which raises many more questions than it solves, and it may turn out in the end to have little or no importance in practical medicine." These words, written in 1925, are, to the reviewer, as sound today as they were over 30 years ago. The caliber of the many other contributions in this volume is high indeed. A good review of the recent studies relating to spinal fluid is contributed by Sweet and of the physiology of increased intracranial pressure by Foley. There is an excellent discussion of the results of treatment of spontaneous subarachnoid hemorrhage (Walsh) and good discussions of the chemistry of myelin. Cawthorne contributes an excellent survey of aural vertigo.

The volume is extremely useful for all interested in the broad aspects of medicine and not only for neurologists. It can be highly recommended. B. A.

BLOOD GROUP SUBSTANCES: THEIR CHEMISTRY AND IMMUNOCHEMISTRY. BY ELVIN A. KABAT, Departments of Microbiology and Neurology, College of Physicians and Surgeons, Columbia University. Pp. 330; 32 ills. New York: Academic Press Inc., 1956. Price, $8.00.

IN this volume, the author has brought together information concerning blood group substances as viewed from the standpoint of quantitative immunochemistry. The subject matter is presented in 10 major sections, including Human Blood Group Factors; Methods Used in Testing for Blood Group Antibodies and Antigens: Sources of Blood Group Substances; Purification of Blood Group Substances: Antibodies to Blood Group Substances and their Biological Effects; and Biological Effects and Uses of Blood Group Substances.

This book is an excellent treatise on a complex subject and complements existing works which deal with the genetic, clinical, and anthropological aspects of the blood groups. It is recommended to all investigators in this and related fields. C. Z.

THE YEAR BOOK OF PEDIATRICS (1957–1958 YEAR BOOK SERIES). Edited by SYDNEY S. GELLIS, M.D., Professor of Pediatrics, Boston University School of Medicine. Pp. 469; 153 ills. Chicago: The Year Book Publishers, Inc., 1957. Price, $7.50.

ONCE again in the Year Book of Pediatrics, 1957–1958 series, the editor has maintained

the same high standard of quality as in preceding volumes. The articles reviewed are well-chosen and cover a wide range of subjects in the field of pediatrics.

One of the most valuable features of the Year Book is the comments made on controversial subjects by the editor or recognized authorities. Through these editorial comments the reader is given a comprehensive review which is not possible by the abstracting of a single article.

This book is an excellent reference source for those engaged in all aspects of pediatrics.

R. O.

EDUCATING SPASTIC CHILDREN: THE EDUCATION AND GUIDANCE OF THE CEREBRAL PALSIED. BY F. ELEANOR SCHONELL, M.A., PH.D., Formerly Research Fellow, University of Birmingham Department of Pediatrics and Child Health. Pp. 242; 18 ills. New York: Philosophical Library, Inc., 1956. Price, $6.00.

THE purpose of this book is stated as, "to provide first hand information of an education and psychological kind" for those working with the cerebral palsied. In the author's material it is found to occur about once in every thousand children of school age. The author points out that there is very little published material giving results of research work of a psychological nature. She shows that 51% had I.Q.'s over 70 and she emphasizes the part that factors other than intelligence play in a child's performance.

The book describes what this group of cases is. Then there are described the results of the survey done in England on the intellectual capacity of these handicapped youngsters. In another part of the book some practical applications of her work are given. It was found that severely handicapped, educatable, palsied children, given the opportunity under proper circumstances show good progress in reading, writing and arithmetic. There is also a good discussion of some of the social and psychological aspects of the problem. The book is well written and illustrated including case reports. It is recommended for all who are interested in the education of the child with cerebral palsy.

S. G.

Current Medical Research. A Reprint of the Articles in the Report of the Medical Research Council for the year 1955–56. Pp. 56. London: Her Majesty's Stationery Office, 1957. Price, 2s. 6d.

The Story of Peptic Ulcer. BY RICHARD D. TONKIN, M.D., F.R.C.P., Westminster Hospital, London. Pp. 71; 67 ills. Philadelphia and London: W. B. Saunders Co., 1957. Price, $2.25.

THE high incidence of ulcer and the need for education of the patient so affected led Dr. Tonkin to create this simplified, entertaining story of peptic ulcer and the people who have them. The illustrations by Hellier will delight any depressed dyspeptic. The few minutes it takes to read this book will be rewarding.

S. L.

Animals Parasitic in Man. BY GEOFFREY LAPAGE. Pp. 320; 75 ills. Baltimore: Penguin Books, Inc., 1957. Price, $.95.

Basic Pharmacology for Nurses. BY JESSIE E. SQUIRE, B.A., R.N., M.ED., Instructor, Hayward-Fairmont School of Vocational Nursing, Hayward, Calif. Pp. 255; 8 ills. St. Louis: C. V. Mosby Co., 1957. Price, $3.50.

THIS is a convenient, well prepared outline of pharmacology for students of nursing. General principles, calculations, measurements, dosages, and various practical points concerning syringes are covered in the first fourteen chapters. The various drugs are then grouped for consideration under the major systems or organs which they affect. A special chapter is devoted to drug legislation. A helpful glossary is included. This outline should prove to be adequate for most student nurses.

R. S.

Neurologia Basica: Semiologia Clinica Propedeutica. BY DR. GUSTAVO F. POCH, Subchief in Neurology, Hospital Ramos Mejia, Buenos Aires, Argentina. Pp. 253; 55 ills. Buenos Aires: Lopez & Etchegoyen, 1957.

A BRIEF description of the most important signs, symptoms and syndromes in neurology that should prove useful in the clinical teaching of medical students.

C. B.

Fear: Contagion and Conquest. BY JAMES CLARK MALONEY, M.D. Pp. 140. New York: Philosophical Library, Inc., 1957. Price, $3.75.

IN order to achieve healthy growth, the individual needs unconditional material love from a motherly mother. The proper measure of maternal love means neither too much nor too little loving support for the child at any stage of its development.

Fear and tensions are contagious. Healthy mothers transferred to an unhealthy culture produce unhealthy children while unhealthy mothers, comfortable in their environment, produce healthier children.

This book is an oversimplication of the complex problem of ego developments. The psychic state of the infant itself is ignored. Cultural factors influencing the mother are touched upon.

M. B.

Bedside Diagnosis. By Charles Seward, M.D., F.R.C.P. (Edin.), Physician, Royal Devon and Exeter Hospital. 4th ed. Pp. 430; illustrated. Baltimore: The Williams & Wilkins Co., 1957. Price, $5.00.

Again within the period of ten years the author has revised and rewritten for the fourth time a concise and succinct classification of human symptoms. Basic symptoms (cough, dyspnea, and the like) form chapter titles and are discussed according to physiology, anatomy, and differential diagnosis. By keeping his text up-to-date, the author has made available to the practitioner and student a handy catalogue of diagnoses which can be arrived at in a logical manner.

W. S.

How to Write Scientific and Technical Papers. By Sam F. Trelease, Ph.D., Columbia University. 3rd ed. Pp. 185. Baltimore: The Williams & Wilkins Company, 1958. Price, $3.25.

This small volume is a *vade mecum* for writers on scientific topics. Under the present title, it has been successful in two earlier editions. Its predecessor, *Preparation of Scientific and Technical Papers*, was first published by Williams & Wilkins in 1925, and went through two other editions. The value of this book is reflected by the demand for successive revisions over the past 33 years.

A real "how to" book, it aims to guide the scientist to prepare sound papers or reports on technical subjects in good English and with a minimum of effort. Seven clearly written chapters outline the task, from seleting a research problem to proofreading galleys and page proofs. A helpful bibliography and index complete the work. This useful book is recommended for all who write scientific and technical papers.

F. R

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